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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/24, C07K 16/24, C12N 15/62, 5/10, A61K 38/20, 48/00

A1

(11) International Publication Number: WO 97/00321

(43) International Publication Date: 3 January 1997 (03.01.97)

(21) International Application Number: PCT/AU96/00360

(22) International Filing Date: 14 June 1996 (14.06.96)

(30) Priority Data:

| | | |
|---|---|---|
| PN 3502 | 14 June 1995 (14.06.95) | AU |
| PN 6244 | 27 October 1995 (27.10.95) | AU |

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(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*

(54) Title: IMMUNE RESPONSE MODULATORS AND USES THEREFOR

(57) Abstract

The present invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a sequence encoding, an ovine IL-5 or IL-12 cytokine molecule. The invention further provides recombinant isolated ovine IL-5 and IL-12 polypeptides which are useful as immune response modulators in livestock animals.

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# IMMUNE RESPONSE MODULATORS AND USES THEREFOR

The present invention relates generally to recombinant polypeptides having ovine cytokine properties and to genetic sequences encoding same. More particularly, the present invention is directed to recombinant ovine interleukins and specifically interleukin-5 (IL-5) and interleukin-12 (IL-12) and their use as immune response modulators, especially in vaccine compositions.

Bibliographic details of the publications referred to in this specification by author are collected at the end of the description. Sequence Identity Numbers (SEQ ID Nos.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification and the claims that follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating research into the medical and veterinary fields. Cytokine research is of particular importance, especially as these molecules regulate the proliferation, differentiation and function of a variety of cells such as cells involved in mediating an immune response. Administration of recombinant cytokines or regulating cytokine function and/or synthesis is becoming, increasingly, the focus of medical research into the treatment of a range of disease conditions in humans and animals.

Cytokines are the hormones of the immune system which control and determine the nature of the immune response (Balkwill and Burke, 1989). Examples of cytokines include interleukins which primarily effect the functional activity of the lymphocytes involved in specific cell-mediated and antibody responses; colony stimulating factors which regulate the

maturation of precursor cells into macrophages, granulocytes, mast cells and lymphocytes which are involved in innate resistance to many pathogens (Metcalf, 1987); the interferons, which, in addition to their direct anti-viral action, also stimulate antibody synthesis, the activity of natural killer cells and the antimicrobial activity of macrophages and neutrophils (Bielefeldt Ohmann *et al.*, 1987). All these molecules have the potential to alter the disease resistance and immune responsiveness of animals to a wide variety of infectious diseases and vaccines.

Much research has been undertaken into the use of cytokines to augment the immune response and to enhance the immunocompetence of the host to eliminate foreign pathogens. However, despite the discovery and availability of a range of cytokines and other secreted regulators of cell function, comparatively few cytokines are used directly or targeted in therapeutic regimens, especially in animals. One reason for this is the pleiotropic nature of many cytokines. For example, interleukin-11 (IL-11) is a functionally pleiotropic molecule capable of inducing multipotential haemopoietin progenitor cell proliferation, enhancing megakaryocyte and platelet formation, stimulating acute phase protein synthesis and inhibiting adipocyte lipoprotein lipase activity.

Another difficulty confronted in cytokine research is that much of the work has been conducted in human and murine systems. As a consequence, far less is known of the role of animal cytokines in the regulation of the immune system.

There is a need, therefore, for a detailed elucidation of the immune response in large animals in order to facilitate an understanding of the effector mechanisms required to confer protection to livestock and other animals of commercial, environmental or domestic importance against various disease conditions. This will also provide more efficacious vaccines and the development of veterinary compositions for livestock animals to protect same against infection especially when the animals are in an immunocompromised state such as stress due to overcrowding and during transport, changes in climate and following early weaning. The commercial importance of such formations, especially in the livestock industry are clearly evident, such as in increased production of meat and wool. In work leading up to the present

invention, the inventors sought to clone ovine cytokine genes. Two cytokines were targeted, IL-5 and IL-12.

IL-5 is a potent growth promoter of early haemopoietic progenitor cells. It also promotes the generation of cytotoxic cells from thymocytes and murine IL-5 stimulates the production and secretion of IgM and IgA by B cells in synergism with bacterial endotoxins (Sonada *et al.*, 1992). Secretory IgA antibodies directed against specific virulence determinants of infecting organisms play an important role in overall mucosal immunity. IL-5 is also a specific stimulator of eosinophil differentiation as well as a selective chemoattractant and eosinophil activation factor.

IL-12 is a heterodimeric cytokine composed of a 40-kDa subunit (p40) disulfide-linked to a 35-kDa subunit (p35) (Kobayashi *et al* 1989); (Stern *et al* 1990). It induces the production of IFN-γ by T and NK cells, stimulates the proliferation of activated T and NK cells and enhances the specific and non-specific cytolytic lymphocyte responses. Accumulating evidence suggests that the lack of effective protection against infectious pathogens may result from the selective activation of T cells with an aberrant cytokine profile. Generally, protection against intracellular bacteria and viruses requires a Th1-type response. IL-12 is the critical cytokine that drives differentiation of naive cells to the Th1 subset resulting in the Th1-type immune response. Thus, IL-12 plays a vital role in inducing protective effector mechanisms against bacterial and viral infections.

In accordance with the present invention, genetic sequences encoding ovine IL-5 and the 35 kDa and 40 kDa subunits of IL-12 have been cloned. The availability of recombinant forms of these two important cytokines will now permit the development of therapeutic and vaccine compositions to enhance the immunoresponsiveness of host animals.

Accordingly, one aspect of the present . vention relates to an isolated nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a nucleotide sequence encoding, an ovine cytokine or a functional or immunologically interactive

homologue, analogue or derivative thereof, wherein said ovine cytokine is IL-5 or IL-12 or a polypeptide subunit of IL-12, or is a fusion cytokine between different subunits of IL-12.

Hereinafter references to "IL-12" or "ovine IL-12" shall be taken to include all possible monomeric, dimeric or other multimeric forms comprising the 35 kDa or 40 kDa polypeptide subunits, including heterodimers and homodimers comprising same. References herein to "IL-12" shall also be taken to include all possible fusion cytokines between the 35 kDa and the 40 kDa polypeptide subunits of ovine IL-12. In a particularly preferred embodiment however, references contained herein to "IL-12" indicates a heterodimer formed between the 35 kDa and 40 kDa polypeptide subunits.

The nucleotide sequence of the cloned cytokines will most preferably include the sequences set forth in SEQ ID No: 1 or SEQ ID No: 3 for IL-5, SEQ ID No: 5 or SEQ ID No: 7 for the 35 kDa subunit of IL-12, or SEQ ID No: 9 for the 40 kDa subunit of IL-12, or a homologue, analogue or derivative thereof including any single or multiple nucleotide substitutions, deletions and/or additions thereto.

In a related embodiment of the present invention there is provided an isolated DNA molecule which:

(i) encodes a molecule having interleukin activity;

(ii) is capable of hybridising under at least medium stringency conditions to one or more of the nucleotide sequences set forth in SEQ ID Nos: 1,3,5,7, or 9 or a complementary sequence or a homologue, analogue or derivative thereof; and

(iii) wherein said interleukin comprises an amino acid sequence corresponding to all or a part of one or more of the amino acid sequences set forth in SEQ ID Nos: 2,4,6,8, or 10 or having greater than 70% similarity thereto.

In a preferred embodiment, the present invention provides an isolated DNA molecule which:

(i) encodes a molecule having IL-5 activity;

(ii) is capable of hybridising under at least medium stringency conditions to all or part of SEQ ID NO: 1 or SEQ ID NO: 3, or a complementary sequence thereof; and

(iii) said interleukin comprises an amino acid sequence corresponding to SEQ ID No: 2 or SEQ ID No: 4 or having 70% or greater similarity thereto.

In a related preferred embodiment the present invention provides an isolated DNA molecule which:

(i) encodes a molecule having IL-12 activity;

(ii) is capable of hybridising under at least medium stringency conditions to one or more of the nucleotide sequences set forth in SEQ ID NO: 5 or SEQ ID NO: 7 or SEQ ID NO: 9 or a complementary form or a homologue, analogue or derivative thereof; and

(iii) wherein said interleukin comprises an amino acid sequence corresponding to all or a part of one or more of the amino acid sequences set forth in SEQ ID No: 6 or SEQ ID No: 8 or SEQ ID No: 10 or having greater than 80% similarity thereto.

These embodiments of the present invention are not intended to cover nor do they cover human or mouse IL-5 or IL-12.

For the purposes of defining the levels of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which are incorporated herein by reference where the washing step at paragraph 11 is considered herein to be high stringency. A high stringency wash is defined herein to be 0.1-0.2xSSC, 0.1% w/v SDS at 55-65°C for 20 minutes and a medium level of stringency is considered herein to be 2xSSC, 0.1% w/v SDS at ≥ 45°C for 20 minutes. The alternative conditions are applicable depending on concentration, purity and source of nucleic acid molecules.

In a more particularly preferred embodiment, the present invention provides an isolated nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which

encodes an ovine IL-5 and having a nucleotide sequence substantially as set forth in SEQ ID NO: 1 or SEQ ID NO: 3, or a homologue, analogue or derivative thereof. In another embodiment, the present invention provides an isolated nucleic acid molecule which encodes, or is complementary to a nucleic acid molecule which encodes a 35 kDa subunit of ovine IL-12 and having a nucleotide sequence substantially as set forth in SEQ ID NO: 5 or SEQ ID NO: 7, or a homologue, analogue or derivative thereof. In yet another embodiment, the present invention provides an isolated nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes a 40 kDa subunit of ovine IL-12 and having a nucleotide sequence substantially as set forth in SEQ ID NO: 9 or a homologue, analogue or derivative thereof.

The term "homologue" as used hereinafter, in relation to a variant genetic sequence, refers to a gene which encodes a polypeptide which retains its function as an interleukin molecule or subunit of same, although said polypeptide may contain amino acid substitutions, deletions and/or additions. The term "homologue" in relation to a variant polypeptide refers to a polypeptide containing amino acid substitutions, amino acid deletions and/or amino acid additions which do not affect the function of the polypeptide. Furthermore, amino acids may be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic moment or antigenicity, and so on. The present invention clearly extends to homologues of ovine IL-5 or IL-12 genetic sequences.

The term "analogue" as used hereinafter in reference to a nucleic acid molecule, shall be taken to refer to a variant genetic sequence which is functionally equivalent to a genetic sequence which encodes or is complementary to a genetic sequence which encodes an ovine IL-5 or ovine IL-12 polypeptide, but which contains certain non-naturally occurring or modified residues. Similarly, the term "analogue" when used in relation to a polypeptide molecule shall be taken to refer to a variant polypeptide which is functionally equivalent to an ovine IL-5 or an ovine IL-12 polypeptide, but which contains certain non-naturally occurring or modified residues.

Hereinafter, reference to "derivatives" includes mutants, parts or fragments of genetic sequences which encode or are complementary to genetic sequences which encode ovine IL-5 or IL-12 polypeptide subunits. The term "derivative" in relation to an ovine IL-5 or IL-12 polypeptide shall be taken to refer hereinafter to mutants, parts or fragments of the complete IL-5 or IL-12 polypeptide subunits comprising the functional ovine interleukin. It is understood by the skilled person in the art that a "derivative" of a nucleic acid molecule or a polypeptide molecule may not have the same physiological activity as the genetic sequence or polypeptide from which it was derived, however it is useful in the isolation of related genetic sequences or polypeptides, or in modifying gene expression, for example by antisense or ribozyme technology, or in the production of useful immunoreactive molecules, for example the production of useful subunit vaccines.

In accordance with the present invention, by "nucleic acid molecule" is meant a single or double stranded sequence of ribonucleotides or deoxyribonucleotides which encode, or are complementary to a sequence which encodes, an ovine IL-5 and/or IL-12 or their derivatives. The nucleic acid molecule may be genomic DNA, cDNA or a synthetic DNA sequence or a derivative thereof. The derivatives may be functional in that they exhibit at least one property or function attributed to IL-5 or IL-12 or are immunologically interactive with antibodies to at least one region of IL-5 or IL-12. The nucleic acid molecule of the present invention is generally in isolated form but the present invention extends to the nucleic acid molecule integrated into a genome or other nucleic acid molecule.

A further aspect of the present invention provides a genetic construct comprising a nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes an ovine IL-5 or ovine IL-12 polypeptide or a homologue, analogue or derivative thereof.

In a particularly preferred embodiment, the present invention provides a genetic construct comprising at least one of the nucleotide sequences set forth in SEQ ID Nos: 1,3,5,7 or 9 or a homologue, analogue or derivative thereof.

The genetic constructs of the present invention are particularly useful for the production of recombinant cytokine molecules encoded therein, when introduced into a cell line and under conditions suitable for gene expression to occur. Such conditions will depend upon the cell line and the expression vector used in each case and would be well-known to the person skilled in the art.

Any number of expression vectors can be employed depending on whether expression is required in a eukaryotic or prokaryotic cell. Furthermore, it is well known in the art that the promoter sequence used in the expression vector will also vary depending upon the level of expression required and whether expression is intended to be constitutive or regulated. Examples of eukaryotic cells contemplated herein include mammalian, yeast, insect or plant cells and examples of prokaryotes include *Escherichia coli, Bacillus* sp. and *Pseudomonas* sp. Typical promoters suitable for expression in bacterial cells such as *E. coli* include, but are not limited to, *tac* promoter, the *lacz* promoter, or the phage lambda $\lambda_L$ or $\lambda_R$ promoters.

A still further aspect of the present invention provides a recombinant isolated ovine IL-5 or IL-12 polypeptide or a homologue, analogue or derivative thereof. By "recombinant cytokine" or related term "recombinant molecule" is meant a glycosylated or unglycosylated polypeptide molecule, with or without other associated molecules (eg. lipids) produced by recombinant means such as presence of a DNA molecule in an expression vector in the correct reading frame relative to a promoter and introducing the resultant recombinant expression vector into a suitable host and growing said host under conditions appropriate for expression and, if necessary, transportation of the recombinant protein or its derivative from said host and then purifying the recombinant molecule.

In a particularly preferred embodiment of the present invention, there is provided a recombinant polypeptide comprising a sequence of amino acids which is substantially the same as the amino acid sequence set forth in any one or more of SEQ ID Nos: 2,4,6,8, or 10, or is at least 70% identical to same. The present invention extends to any derivatives of ovine IL-5 or IL-12 polypeptides set forth in SEQ ID NOS. 2, 4, 6, 8 or 10.

Derivatives of ovine IL-5 and IL-12 include single or multiple amino acid substitutions, deletions and/or additions to the molecule. Conveniently, these are prepared by first making single or multiple nucleotide substitutions, deletions and/or additions to the nucleic acid molecule encoding the ovine cytokine. Alternatively, once the amino acid sequence is known, amino acids can be chemically added by established techniques and in any sequence required to give the desired mutant. All such derivatives are encompassed by the present invention.

Amino acid insertional derivatives of the ovine cytokines of the present invention include amino and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Typical substitutions are those made in accordance with Table 1.

Where a derivative ovine cytokine is produced by amino acid substitution, the amino acids are generally replaced by other amino acids having like properties, such as hydrophobicity, hydrophilicity, electronegativity, bulky side chains and the like. Amino acid substitutions are typically of single residues. Amino acid insertions will usually be in the order of about 1-10 amino acid residues and deletions will range from about 1-20 residues. Preferably, deletions or insertions are made in adjacent pairs, i.e. a deletion of two residues and a corresponding insertion of two residues.

**TABLE 1**

**Suitable residues for amino acid substitutions**

| Original Residue | Exemplary Substitutions |
|---|---|
| Ala | Ser |
| Arg | Lys |
| Asn | Gln; His |
| Asp | Glu |
| Cys | Ser |
| Gln | Asn; Glu |
| Glu | Asp |
| Gly | Pro |
| His | Asn; Gln |
| Ile | Leu; Val |
| Leu | Ile; Val |
| Lys | Arg; Gln; Glu |
| Met | Leu; Ile; Val |
| Phe | Met; Leu; Tyr |
| Ser | Thr |
| Thr | Ser |
| Trp | Tyr |
| Tyr | Trp; Phe |
| Val | Ile; Leu; Met |

For convenience and by way of shorthand notation, reference herein to ovine cytokine IL-5 or IL-12 includes reference to any derivatives thereof as contemplated above.

The amino acid variants referred to above may be readily made using synthetic peptide techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Techniques for making substitution mutations at predetermined sites in DNA having known or partially known sequence are well known and include, for example, M13 mutagenesis. The manipulation of DNA sequence to produce variant proteins which manifest as substitutional, insertional or deletional variants are conveniently described, for example, in Sambrook *et al* (1989).

Other examples of recombinant or synthetic mutants and derivatives of the ovine cytokines of the present invention include single or multiple substitutions, deletions and/or additions of any molecule associated with the enzyme such as carbohydrates, lipids and/or proteins or polypeptides.

The recombinant ovine IL-5 and IL-12 molecules contemplated herein will find particular application in the intensive livestock industries such as the live animal export trade, feed-lots and intensive rearing industries. Animals in close containment are subjected to greater environmental challenge with infectious diseases, particularly respiratory infections and are more prone to the immunodepressive effects of stress leading to higher susceptibility to opportunistic pathogens.

Accordingly, in a further aspect of the present invention there is provided a method for the treatment and/or prophylaxis of a livestock animal exposed to or infected with a pathogenic organism, said method comprising administering to said animal an immunoresponsive effective amount of ovine IL-5 and/or ovine IL-12 or a homologue, analogue or derivative thereof for a time and under conditions sufficient to maintain, stimulate or enhance the immunoresponsiveness of said animal.

Preferably, the ovine cytokine is a recombinant molecule. The term "livestock animal" extends to sheep, horses, pigs, cows, donkeys, emus, ostriches, alpacas, camels, deer, goats, amongst other animals, provided that the ovine cytokines are effective in those animals.

Particularly preferred livestock animals are sheep and cows.

Another important application of the cytokines of the present invention is as natural adjuvants for vaccines, particularly for subunit vaccines produced by recombinant DNA technology. In accordance with the present invention, IL-5 and/or IL-12 are used in vaccines to enhance the immunogenicity of antigens, particularly in subunit vaccines. Advances in slow-release technology and the development of live non-pathogenic bacteria and viruses as delivery vectors for these molecules will ensure their cost-effectiveness in sheep and cattle. They may also be used as in nucleic acid vaccination. Accordingly, the present invention extends to a method of enhancing and/or stimulating an immune response to one or more antigens in an animal, said method comprising administering to said animal an immunoresponsive effective amount of IL-5 and/or IL-12.

In a related embodiment, there is contemplated a vaccine comprising an antigen and recombinant ovine IL-5 and/or IL-12 or their derivatives. The vaccine may also comprise one or more pharmaceutically acceptable carriers and/or diluents. The carriers and/or diluents are also required to be acceptable for veterinary use.

The ovine IL-5 and/or IL-12 may also be delivered by genetic means. For example, the recombinant ovine IL-5 and/or IL-12 may be encoded by a genetic construct present in a delivery system such as a virus, yeast, bacterium, protozoan, insect or mammalian cell. The presence of such a delivery system in a target animal will enable delivery of the recombinant ovine cytokine.

According to this embodiment, there is provided a genetic construct comprising a first nucleotide sequence encoding ovine IL-5 or ovine IL-12 or their derivatives and a second nucleotide sequence defining a delivery vehicle. The delivery vehicle is capable of replication in a delivery cell such as a bacterial, yeast, insect, a protozoan animal or a mammalian cell. Generally, the delivery cells would not in normal use be harmful or pathogenic to the target animal. Conveniently, attenuated delivery cells are employed. Particularly useful delivery cells

are bacterial cells, attenuated viruses and particularly suitable delivery vectors are recombinant viral and bacterial vectors.

For example, an attenuated infectious virus is used as a live vaccine. The genetic sequence encoding ovine IL-5 and/or IL-12 or their derivatives is cloned into the viral sequence and the recombinant virus used to infect target animals. The recombinant virus causes infection and replicates in the animal cells resulting in production of the recombinant cytokine. The infecting recombinant virus may subsequently be eliminated after production of an immunomodulating effective amount of the cytokine. A similar protocol is adopted with live bacterial carriers. Alternatively, a recombinant viral vector may be used. A viral vector provides a modified virus capable of infecting a cell but not replicating therein. A viral vector provides a means of introducing a genetic sequence which is transiently capable of expression into the desired cytokine. An "immunomodulating effective amount" is an amount of cytokine sufficient to effect immunomodulation in the target animal, i.e. to enhance the ability of the immune system to develop an effective immune response or to enhance the immunocompetence of the animal or immunogenicity of an antigen which may also be expressed in the genetic vector.

The present invention provides an opportunity to enhance an immune response in animals and in particular livestock animals (such as those described above) by the administration of an ovine IL-5 and/or IL-12 or their derivatives either directly or via their genetic sequences. This is of particular importance since most subunit and synthetic peptide vaccines are only weakly antigenic. The administration of the cytokines may be alone, in combination with an antigen or as a fusion molecule. Administration may be via an attenuated virus, recombinant viral vector nucleic acid vaccine or bacterial vector or may be by administration of the cytokine by, for example, injection or oral ingestion (e.g. in medicated food material).

The present invention extends to a veterinary pharmaceutical composition for use in livestock animals such as to enhance the immune system or accelerate its maturation or improve

its immunocompetence or to facilitate immunomodulation in said animals, said composition comprising recombinant ovine IL-5 and/or IL-12 or their derivatives, recombinant ovine IL-5 and/or IL-12 fused to an antigen or to each other with or without antigen or genetic sequences encoding same in suitable delivery vehicles. Preferably, where the composition comprises a recombinant cytokine, the composition is injected or orally administered. Where the composition comprises genetic material, it is administered as part of a viral vector, live viral vector, live bacterial vector or nucleic acid vaccine.

Conditions in livestock animals for which treatment might be required include infectious disease, cancer, immunosuppression, allergy and to enhance or suppress reproductive systems. Conditions would also include situations where animals are in an immunocompromised state such as during or following stress, due to overcrowding and transport process, changes in climate and early weaning. The administration of the cytokine molecules may also promote growth and/or early maturation. The animal to be treated and the cytokine in the composition might be "homologous" in the sense that both are of the same species, i.e. both ovine species or may be "heterologous" where the ovine cytokine is effective in another animal. The compositions may also contain other active molecules such as antibiotics or antigen molecules. Combinations of cytokine molecules with antigen molecules may increase the efficacy of vaccines.

The present invention, therefore, provides a veterinary pharmaceutical composition comprising an immunomodulatingly effective amount of ovine IL-5 and/or IL-12 or their derivatives or genetic sequences capable of expressing same and one or more carriers and/or diluents acceptable for veterinary use.

The active ingredient(s) of the pharmaceutical composition is/are contemplated to exhibit excellent activity in stimulating, enhancing or otherwise facilitating an immune response in an animal species and in particular a livestock animal when administered in an amount which depends on the particular case. The variation depends, for example, on the cytokine and, in some cases, the antigen involved in stimulating the immune response. For example, from about

0.5 μg to about 100 μg of a particular cytokine which may be combined with other cytokines, per kilogram of body weight per day may be required. Dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered in one or more of daily, weekly or monthly or in other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The active compound may be administered by injection or by oral ingestion in any convenient manner or may be administered via a genetic sequence such as in a viral or bacterial vector or a nucleic acid vaccine.

The active compounds may also be administered in dispersions prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for parenteral administration include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient(s) into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Carriers and/or diluents suitable for veterinary use include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the composition is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The latter is particularly contemplated as far as the present invention extends to multivalent vaccines or multi-component cytokine molecules.

The pharmaceutical veterinary compositions of the present invention may comprise in addition to IL-5 and/or IL-12 or their derivatives, one or more other active compounds such as antigens and/or immune stimulating compounds.

The cytokine may also be delivered by a live delivery system such as using a bacterial expression system to express the cytokine protein in bacteria which can be incorporated into gut flora. Alternatively, a viral expression system can be employed or incorporated into a BCG vaccine. In this regard, one form of viral expression is the administration of a live vector generally by spray, feed or water where an infecting effective amount of the live vector (e.g. virus or bacterium) is provided to the animal. Another form of viral expression system is a non-replicating virus vector which is capable of infecting a cell but not replicating therein. The

non-replicating viral vector provides a means of introducing genetic material for transient expression into a cytokine. The mode of administering such a vector is the same as a live viral vector.

The present invention extends to antibodies raised against ovine IL-5 or IL-12. The antibodies may be monoclonal or polyclonal and may be used for developing enzyme-immunosorbent assays for the rapid diagnosis of infectious diseases of livestock animals. According to this embodiment, there is provided an antibody preparation comprising antibodies or derivatives thereof, immunointeractive with either IL-5 or IL-12 or derivatives thereof.

Immunoassays are useful in detecting the presence of IL-5 and/or IL-12 in a target animal.

A wide range of immunoassay techniques may be such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These methods may be employed for detecting IL-5 and/or IL-12. By way of example only, an IL-5 or IL-12-specific antibody is immobilised onto a solid substrate to form a first complex and a biological sample from an animal to be tested for the presence of IL-5 or IL-12 brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-IL-5/IL-12 secondary complex, a second IL-5/IL-12 antibody labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing sufficient time for the formation of a tertiary complex of antibody-IL-5/IL-12-antibody. Any unreacted material is washed away, and the presence of the tertiary complex is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparison with a control sample containing known amounts of hapten. Variations of this assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and then added simultaneously to the bound antibody. These techniques are well known to those

skilled in the art, and the possibility of minor variations will be readily apparent. The antibodies used above may be monoclonal or polyclonal.

The solid substrate is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining complex is then exposed to the light of the

appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

The present invention is further described by reference to the following non-limiting Figures and Examples.

In the Figures:

**Figure 1** is a schematic representation showing the nucleotide sequence of the exons and the intron/exon splice junctions of the ovine IL-5 gene [SEQ ID NO:1].

**Figure 2** is a schematic representation showing the alignment of the deduced amino acid sequence of ovine IL-5 [SEQ ID NO:2] with human and mouse IL-5 polypeptides.

**Figure 3** is a schematic representation showing an alignment of the deduced partial amino acid sequence of ovine IL-12 35kDa subunit [SEQ ID NO:6] with human and mouse IL-12 polypeptide molecules. The symbol (•) indicates that the amino acid is missing.

**Figure 4** is a schematic representation showing the construction of the expression vector pCI-neo/p35, which expresses ovine IL-5 as a fusion protein with a polyhistidine (6xHis) polypeptide.

**Figure 5** is a schematic representation showing the construction of a vector which expresses ovine IL-5 as a fusion protein with glutathione-S-transferase in a pGEX bacterial expression vector.

**Figure 6** is a schematic representation showing the construction of a vector which expresses 35 kDa ovine IL-12 as a fusion protein with glutathione-S-transferase in a pGEX bacterial expression vector.

**Figure 7** is a schematic representation showing the construction of a vector which expresses 40 kDa ovine IL-12 as a fusion protein with glutathione-S-transferase in a pGEX bacterial expression vector.

**Figure 8** is a schematic representation showing the expression vector pCI-neo/IL-12 which co-expresses the 35 kDa and 40 kDa ovine IL-12 subunits under the control of the CMV I.E promoter/enhancer sequence.

**Figure 9** is a graphical representation showing the biological activity of recombinant ovine IL-5 (rOvIL-5) in a murine BAF cell (IL-5 dependent cell line) proliferation assay.

SEQ ID NOs referred to herein are summarised in Table 2.

Single and three letter abbreviations used for amino acid residues are shown in Table 3.

**TABLE 2**

**SEQUENCE IDENTITY NUMBERS**

| SEQ ID NO: | SEQUENCE |
|---|---|
| 1 | Nucleotide sequence of exons from ovine IL-5 gene |
| 2 | Amino acid sequence of ovine IL-5 derived from the nucleotide sequence of the genomic clone |
| 3 | Nucleotide sequence of ovine IL-5 cDNA |
| 4 | Amino acid sequence of ovine IL-5 polypeptide derived from nucleotide sequence of cDNA clone |
| 5 | Partial sequence of ovine IL-12 35kDa subunit cDNA |
| 6 | Partial amino acid sequence of ovine IL-12 35kDa subunit |
| 7 | Nucleotide sequence of ovine IL-12 35kDa subunit cDNA |
| 8 | Amino acid sequence of ovine IL-12 35kDA subunit |
| 9 | Nucleotide sequence of ovine IL-12 40kDa subunit cDNA |
| 10 | Amino acid sequence of ovine IL-12 40kDa subunit |
| 11 | Forward primer for cloning ovine IL-5 gene |
| 12 | Reverse primer for cloning ovine IL-5 gene |
| 13 | Forward primer for cloning ovine IL-5 cDNA |
| 14 | Reverse primer for cloning ovine IL-5 cDNA |
| 15 | Forward primer for cloning partial cDNA encoding ovine IL-12 35kDa subunit |
| 16 | Reverse primer for cloning partial cDNA encoding ovine IL-12 35kDa subunit |
| 17 | Forward primer for cloning ovine IL-12 35kDa subunit cDNA |
| 18 | Reverse primer for cloning ovine IL-12 35kDA subunit cDNA |
| 19 | Forward primer for cloning ovine IL-12 40kDA subunit cDNA |
| 20 | Reverse primer for cloning ovine IL-12 40kDA subunit cDNA |

**TABLE 3**

| Amino Acid | Three-letter Abbreviation | One-letter Symbol |
|---|---|---|
| Alanine | Ala | A |
| Arginine | Arg | R |
| Asparagine | Asn | N |
| Aspartic acid | Asp | D |
| Cysteine | Cys | C |
| Glutamine | Gln | Q |
| Glutamic acid | Glu | E |
| Glycine | Gly | G |
| Histidine | His | H |
| Isoleucine | Ile | I |
| Leucine | Leu | L |
| Lysine | Lys | K |
| Methionine | Met | M |
| Phenylalanine | Phe | F |
| Proline | Pro | P |
| Serine | Ser | S |
| Threonine | Thr | T |
| Tryptophan | Trp | W |
| Tyrosine | Tyr | Y |
| Valine | Val | V |
| Any residue | Xaa | X |

## EXAMPLE 1

## CLONING OF OVINE IL-5 GENE

An ovine genomic library constructed in EMBL3 vector was obtained from Clontech, USA. Phage were used to infect *Escherichia coli* strain K802. One hundred thousand plaques were transferred onto nylon filters and screened with a {$^{32}$P}-labelled fragment of human IL-5 cDNA. The filters were washed at 2xSSC, 0.1% (w/v) SDS, 65°C for 20 minutes. Five positive plaques were purified after three rounds of plating and screening. When the phage DNA was used as the template for PCR, all five preparations of DNA gave a strong intense band of approximately 2 kb in size. The phage DNA was used as template in the PCR using the primers as follows:

CTT TCT TTG CCA AAG GCA AAC GC [SEQ ID No: 11] forward primer and

TGG CCC TCA TTC TCA CTG CA [SEQ ID No: 12] reverse primer.

The conditions for PCR were 30 cycles of 94°C for one min, 55°C for two min and 72°C for 2 min. The amplified PCR product was cloned into the *Sma*I site of pUC18 vector and 3 clones were sequenced by dideoxy sequencing using an automatic DNA sequencer.

One of the PCR products from clone 3-1 was chosen for cloning into pUC18 vector. The nucleotide sequence [SEQ ID NO: 1] of the exons of the ovine IL-5 gene is shown in Figure 1. Figure 2 shows the alignment of the deduced amino acid sequence [SEQ ID NO: 2] of ovine IL-5 with human and mouse IL-5. The overall amino acid homology of ovine IL-5 protein with human and mouse IL-5 molecules were 65% and 54%, respectively.

## EXAMPLE 2

## CLONING OF OVINE IL-5 cDNA

RNA from peripheral lymph node cells stimulated for 24 hours with Concanavalin (5µg/ml) was isolated using Trizol (Gibco, BRL) according to the manufacturer's instructions.

RNA (5 μg) was reverse-transcribed to produce single-stranded cDNA, using Superscript RNase H-reverse transcriptase (Gibco- BRL). Ovine IL-5 cDNA sequences were then amplified in a polymerase chain reaction using Taq polymerase (Gibco- BRL) and the following primers:

CGCGGATCCATGCATCTGCGTTTGACCTTG [SEQ ID No: 13] forward primer and

TCAGCTTTCCATGCTCCACTC [SEQ ID No: 14] reverse primer.

The primers were based on the genomic sequence of ovine IL-5 gene set forth in SEQ ID NO: 1. The conditions for PCR were 30 cycles of amplification as follows:

94°C for 30 seconds;

55°C for 30 seconds; and

72°C for 30 seconds.

The amplified DNA was cloned into the *Sfr*I site of pCRSCRIPTSK$^+$ (Stratagene, USA). Four clones were sequenced in both directions using the M13 forward primer and the reverse primer using the Applied Biosystem 373A DNA sequencer. The complete nucleotide sequence of the IL-5 cDNA clone is set forth in SEQ ID NO: 3. The predicted amino acid sequence of full-length ovine IL-5 is set forth in SEQ ID NO: 4.

## EXAMPLE 3

## CLONING OVINE cDNA ENCODING PARTIAL 35 kDa SUBUNIT OF IL-12

### 1. Isolation and culture of ovine alveolar macrophages

A Merino lamb was euthanased and the lungs aseptically removed. The lungs were lavaged with 250 ml of phosphate buffered saline at pH7.3 containing 6 mM EDTA. Approximately 150 ml of this solution was then removed from the lungs *via* a sterile plastic tubing connected to a 50 ml syringe and the cells collected pelleted by centrifugation (500 g for 10 mins). The cells were washed twice in Dulbecco's modified Eagle's medium (Flow, Australia) supplemented with 20 mM Hepes, 9 mM sodium bicarbonate, 2 mM glutamine, 50

μM 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% (v/v) heat-inactivated foetal bovine serum. The cells were resuspended in culture medium and viable cells enumerated by trypan blue exclusion. Cells were found to be greater than 95% macrophages as estimated by microscopic examination. The macrophages were cultured for 5 h at 37°C in 90 mm plastic tissue culture petri dishes ($1 \times 10^7$ cells/dish) containing 12 ml of culture media and 20 μg/ml of lipopolysaccharide (Sigma, USA). The adhered macrophages were washed with PBS and then lysed in 1 ml Trizol (Gibco-BRL).

**2. Reverse transcription - PCR**

RNA from LPS-stimulate ovine alveolar macrophages lysed in Trizol were isolated according to manufacturer's instruction. An amount of 5 μg of RNA was used for first strand complementary DNA synthesis using Superscript RNase H- reverse transcriptase (Gibco-BRL) and PCR performed with Taq polymerase (Gibco-BRL) and the following primers:

CGCGGATCCACCACCTCAGTTTGGCCAGG [SEQ ID No: 15] forward primer and

CGCGGATCCGGCGTGAAGCAGGATGCAGAG [SEQ ID No: 16] reverse primer.

The amplified DNA fragment was subcloned into the BamHI site plasmid pUC18. DNA sequencing by the dideoxy termination method was performed on both strands using the universal and reverse primers.

**3. Cloning results**

The nucleotide sequence of the partial cDNA encoding the 35 kDa subunit of IL-12 is set forth in SEQ ID NO: 5.

Figure 3 shows the alignment of the deduced partial amino acid sequence [SEQ ID NO: 6] of the 35 kDa subunit of ovine IL-12 compared to the bovine, human and mouse IL-12 cytokines. The level of amino acid homology with human and mouse equivalents are 79 and 61%, respectively.

## EXAMPLE 4

## CLONING A FULL-LENGTH cDNA ENCODING THE 35 kDa SUBUNIT OF OVINE IL-12

Ovine alveolar macrophages were lipopolysaccaride (LPS)-stimulated as described in the preceding Examples for 4 hours. Macrophages were subsequently lysed in Trizol (Gibco BRL) and RNA was isolated according to the manufacturer's instructions. RNA (5 μg) was used as a template for first-strand cDNA synthesis using Superscript RNase H- reverse transcriptase (Gibco-BRL). Ovine IL-12 sequences were amplified using the cDNA as a template and the following primers:

CGCCTCGAGATGTGCCCGCTTCGCAGCCTC [SEQ ID No: 17] forward primer and

CGCGGTACCCTAGGAAGAACTCAGATAGCT [SEQ ID No: 18] reverse primer.

The amplified DNA fragment was subcloned into the *Sma*I site of plasmid pUC18. DNA sequencing was performed using the Applied Biosystem 373A DNA sequencer. Both strands were sequenced using the universal and reverse sequencing primers.

The nucleotide sequence of the full-length cDNA encoding the 35 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 7. The predicted amino acid sequence of the 35 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 8.

## EXAMPLE 5

## CLONING A FULL-LENGTH cDNA ENCODING THE 40 kDa SUBUNIT OF OVINE IL-12

Ovine peripheral lymph node cells were cultured with the phorbol ester, phorbol myristate acetate (PMA) at 10 ng/ml and calcium ionophore A23187 (0.5 μg/ml) for 24 hours

and the cells were lysed in TRizol (Gibco BRL) according to the manufacturer's instructions. RNA was isolated and PCR performed with the following primers:

CGCGGATCCATGCACCCTCAGCAGTTGGTC [SEQ ID NO: 19] forward primer and

CGCGTCGACACTGCAGGACACAGATGCCCA [SEQ ID No: 20] reverse primer.

The PCR product was cloned into the *Sma*I site of the plasmid pUC18 and four clones were sequenced with the M13 universal and reverse sequencing primers using the Applied Biosystem 373A DNA sequencer. The sequencing reactions were performed using the PRISM™ Dye Deoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems). Using these primers approximately 350-400 nucleotide sequence could be determined from the 5′ and 3′ ends of the cDNA.

**1. Subcloning step**

One of the full-length cDNAs encoding the 40 kDa subunit of ovine IL-12 was digested with *Sau*3AI and *Eco*RI and a DNA fragment of approximately 400bp was subcloned into the *Bam*HI/*Eco*RI site of pUC118. This subclone was sequenced using the universal and reverse primers as described above.

The complete nucleotide sequence of the full-length cDNA encoding the 40 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 9. The predicted amino acid sequence of the full-length 40 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 10.

## EXAMPLE 6

## IL-5 BIOASSAY USING BAF MOUSE CELL LINE

IL-5 dependent murine BAF cells were grown in the presence of murine X63 cell line supernatant [5%(v/v)] as an IL-5 source, in DMEM /10%(w/v)FCS. A well-grown cell

culture, grown in a 75cm$^3$ flask was decanted into a 50 ml centrifuge tube and centrifuged at 1200 rpm for 10 mins. Cells were then resuspended in 10 % (v/v) DMEM and recentrifuged at 1200rpm for 10 mins. Cell washes were repeated twice to remove exogenous IL-5 and finally resuspended to a concentration of 5 X $10^4$ cells /ml. Recombinant ovine IL-5 protein generated from the expression system was titrated in triplicate across a 96 well tissue culture plate then 100 µL of the washed BAF cell suspension was added to a final concentration of 5 X $10^3$ cells/well . Murine IL-5 was used as a positive control for cell proliferation. The cell cultures were incubated in 5%(v/v) $CO_2$ at 37$^0$C for 2 days then pulsed for 8-18 hr with tritiated thymidine, harvested and counted to determine the amount of radioactivity incorporated.

## EXAMPLE 7

## CYTOKINES

Recombinant ovine IL-5 and IL-12 are prepared basically as described for the preparation of recombinant ovine IL-1β in International Patent Application No. PCT/AU91/00419. Briefly, IL-5 and IL-12 are prepared as follows:

**1. Construction of ovine interleukin-5 expression vector**

The ovine IL-5 cDNA encoding the mature IL-5 protein was obtained as described in Example 2.

In one embodiment, the coding sequence for the mature form of the IL-5 gene was PCR-amplified using a thermostable polymerase and introduced into the polylinker of an expression vector belonging to the pQE-30 series of vectors, wherein the vector was selected such that the reading frame of IL-5 mature form coding sequence was in-frame with the reading frame of polyhistidine (6xHis) contained therein. The resultant expression construct was designated pQE-30-IL-5. Figure 4 is a schematic illustration of the expression construct pQE-30-IL-5. The pQE-30-IL-5 expression construct was introduced

into *E.coli* strain DH5α, where induction of the P5 promoter results in high level expression of a fusion protein comprising the polyHis and IL-5 polypeptides.

In an alternative embodiment, the cDNA insert is ligated into the multiple cloning site of an expression vector belonging to the pGEX series of vectors, wherein the vector is selected such that the reading frame of IL-5 is in-frame with the reading frame of glutathione-s-tranferase contained therein (Smith and Johnson, 1988). The IL-5 open reading frame is cloned immediately downstream of the thrombin cleavage site, to produce an in-frame fusion. The recombinant plasmid is designated pGEX-IL-5. Figure 5 is a schematic illustration of the expression plasmid pGEX-IL-5 showing the site of thrombin cleavage of the fusion protein. Transformants of *Escherichia coli* strain JM109 are then produced.

**2. Expression and affinity purification of recombinant interleukin-5**

To express IL-5 under the control of the P5 promoter, bacterial colonies transformed with pQE-30-IL-5 (see above, Example 7.1) were picked and cultured overnight at 37°C in LB growth medium [1%(w/v) tryptone, 0.5%(w/v) yeast extract, 1% (w/v) NaCl] supplemented with ampicillin (50μg/ml). Flasks containing 1L of LB growth medium and ampicillin (50μg/ml) and a 1:50 inoculum of overnight cultures were shaken at 37°C. After 2 hours, the P5 promoter of the expression construct pQE-30-IL-5 was induced with IPTG to a final concentration of 1mM and incubated for a further for two hours.

To purify the recombinant IL-5 protein, 2ml of a 50% slurry of Ni-NTA resin (Clontech) were first equilibrated with PBS. The bacterial cells expressing the polyhistidine-IL-5 fusion protein were recovered by centrifugation at 4000 g for 10 min and the pellet sonicated in 2.5% (v/v) Zwittergent (Sigma, product No T7763). The sonicate was mixed with the Ni-NTA slurry for 30min. Unbound proteins were removed from the supernatant fraction following centrifugation at 800 g. Recombinant IL-5 was eluted from the Ni-NTA slurry with 1 bed volume of 50mM imidazole. Multiple eluants were collected

to maximize yield.

When IL-5 is expressed under the control of the *tac* promoter, overnight cultures of the pGEX-IL-5 plasmid are diluted in 250ml of Luria Broth (10g/L bacto-tryptone, 5g/L yeast extract, 10g/L NaCl) or Terrific Broth (16.43g/L $K_2HPO_4.3H_20$, 2.31g/L $KH_2PO_4$, tryptone 12g/L, yeast 24g/L, glycerol 4ml/L) containing 100/μg/ml ampicillin. The cultures are grown for 2h at 37°C before adding IPTG (isopropyl- β-thiogalactopyranoside) to 0.2mM (or as indicated in the legend). Induction of the *tac* promoter results in high-level expression of a fusion protein between GST and IL-5. After 4h, the cultures are harvested and centrifuged. The pellets are weighed and resuspended in the appropriate volume of buffer (50mM Tris/HCl, pH7.5; 10ml/g of wet weight of pellet). The cells are lysed on ice by sonication and then centrifuged.

To purify the recombinant GST-IL-5 fusion protein, the supernatant is loaded onto a 5ml glutathione Sepharose column (sulphur-linkage,Sigma). The flow through is retained and the column in then washed thoroughly with at least 5 bed volumes of 50mM Tris/HCl, pH7.5. The recombinant IL-5 protein is eluted either as a fusion product with 5mM glutathione or as free IL-5 by cleavage with human thrombin (10U/ml; ICN) at room temperature for 1h. The eluted proteins are analysed by electrophoresis on a 15% (w/v) SDS/polyacrylamide gel and visualised by staining with 0.05% (w/v) Coomassie Brilliant Blue R.

**3. Expression and purification of recombinant ovine IL-12 polypeptides**

The cDNAs encoding the mature form of the 35 kDa and 40 kDa subunits of ovine IL-1β are cloned into the multiple cloning site of an expression vector belonging to the pGEX series of vectors, wherein the vector is selected such that the reading frame of each IL-12 cDNA sequence is in-frame with the reading frame of glutathione-s-tranferase contained therein (Smith and Johnson, 1988), to produce the expression plasmids pGEX-IL12a and pGEX-IL12b, respectively. Figures 6 and 7 are schematic illustrations of the expression plasmids pGEX-IL12a and pGEX-IL12b respectively, showing the sites of

thrombin cleavage of the fusion protein. Induction of the tac promoter of the expression plasmids pGEX-lL12a and pGEX-IL12b results in high level expression of a fusion protein in each case.

Affinity chromatography of the fusion proteins on a glutathione-Sepharose column, followed by cleavage with thrombin, yields the free form of the mature IL-12 35 kDa and 40 kDa subunits. Alternatively, elution from the column with glutathione yields GST-IL12 fusion proteins with approximate molecular weights of 61kDa for the 35 kDa IL-12 subunit) and 66 kDa (for the 40 kDa IL-12 subunit).

**4. Expression of recombinant ovine IL-12 heterodimer**

The cDNA encoding the 35kDa subunit of ovine IL-12 is cloned into the multiple cloning site of the mammalian expression vector pCI-neo, between the CMV I.E enhancer/promoter/ intron sequence and the SV40 late polyadenylation sequence, to produce the intermediate expression vector pCI-neo/p35. Expression of the 35 kDa IL-12 polypeptide in pCI-neo/p35 is under the control of the CMV I.E enhancer/promoter and chimeric intron sequences

The cDNA encoding the 40kDa subunit of ovine IL-12 is cloned into the multiple cloning site of the mammalian expression vector pSI, between the CMV I.E enhancer/promoter/intron and the SV40 late polyadenylation sequence, to produce the intermediate vector pSI/p40. Expression of the 40 kDa IL-12 polypeptide in pSI/p40 is under the control of the CMV I.E enhancer/promoter and chimeric intron sequences.

An expression cassette comprising the 40 kDa IL-12 subunit coding sequence, together with the CMV I.E. enhancer/promoter, chimeric intron and SV40 polyadenylation sequence is removed from the pSI/p40 construct nd introduced into a compatible site in the pCI-neo/p35 vector construct to create a dual construct, designated pCI-neo/IL-12, that contains open reading frames encoding both the 35 kDa and 40 kDa polypeptides in their

respective expression cassettes and under the operable control of the CMV I.E promoter/enhancer sequences. Figure 8 is a schematic representation of the dual expression construct pCI-neo/IL-12.

The construct pCI-neo/IL-12 is introduced into a mammalian cell line for transient or stable expression. Expression is constitutive for both genes. The IL-12 heterodimer forms from the subunits and is secreted into the culture medium.

**5. Protein assays**

Protein concentrations are estimated by the Bradford dye assay (Biorad) using bovine serum albumin as standard.

## EXAMPLE 8

## BIOASSAY OF RECOMBINANT IL-5

Recombinant IL-5 (rOvIL-5) was prepared and purified from cells transformed with the expression construct pQE-30-IL-5 as described in Example 7 and subsequently assayed for biological activity using the BAF mouse cell line bioassay described in Example 6. As shown in Figure 9, significant biological activity above that observed for control samples, was detected for rOvIL-5 produced using the expression vector pQE-30-IL-5 (Figure 4), when the recombinant polypeptide was present in the assay samples at a concentration greater than 1/32 dilution.

## EXAMPLE 9

## VACCINE PREPARATIONS

The recombinant *Taenia ovis* fusion protein GST-45W (Johnson et al., 1989) is used

as the model antigen in all studies. Vaccines are formulated with 50μg/dose of GST-45W and 0, 10 or 100μg/dose of recombinant IL-5 and/or 35 kDa IL-12 and/or 40 kDa IL-12 polypeptides in either phosphate buffered saline (PBS) or the conventional adjuvants Quil A (1 or 5mg/ml), incomplete Freund's adjuvant (IFA; 1:1, oil:water) and aluminium hydroxide (6 mg/ml). Sheep are injected intramuscularly (i/m) (1ml) into the left hind leg for the primary inoculation and 4 weeks later boosted with an i/m injection of the same vaccine preparation into the right hind leg.

## EXAMPLE 10
## SEROLOGY

Sera are collected from all animals before the primary inoculations and then at weekly intervals until 4 weeks post secondary inoculation. Sera are stored at -20°C until assayed for antibodies to 45W using the enzyme immunoassay (EIA) described below. Pre-bleed sera from all sheep are screened for antibodies to 45W prior to the commencement of experiments and any animals demonstrating significant antibody levels to 45W (EIA OD > 0.2 at 1/300 serum dilution) were excluded. For the EIA, either recombinant 45W, thrombin cleaved and purified from the GST moiety or GST-45W (as indicated), is bound to 96-well microtitre plates (Nunc Maxisorb) by incubating 0.2μg per well in 100μl of 50mM carbonate buffer (pH 9.6) for 20hrs at 20°C. The plates are then post-coated (1hr at 20°C) with 100μl per well of phosphate buffered saline (PBS: 0.9% w/v, pH 7.2) containing 1% (w/v) sodium casein. After 4 washes with phosphate buffered saline containing 0.05% v/v Tween 20 (PBST), 100μl of serial dilutions of serum samples are added to the wells for 1hr at 20°C. The plates are then washed 4 times with PBST before the addition of 100μl per well of a 1/1000 dilution of horseradish peroxidase conjugated anti-ovine IgG monoclonal antibody (VET05, Silenus, Australia) in PBST for 1 hr at 20°C. Plates are washed 5 times with PBST and 100μl of tetra-methyl benzidine (TMB) substrate (Bos et al, 1981) added to each well for 30min at 20°C before the reaction is stopped by the addition of 50μl of 0.5M $H_2SO_4$ per well and the absorbance read at 450nm.

## EXAMPLE 11

## ADJUVANT ACTIVITY OF RECOMBINANT IL-5

Sheep are randomly allocated into 12 groups of 5 animals. Serum samples were collected prior to first vaccination and then at weekly intervals until 4 weeks post secondary vaccination. Serum antibody levels to purified 45W are quantified by EIA. All vaccine formulations are standardised to contain 50μg of GST-45W per dose. The addition of at least 10-100μg of recombinant IL-5 to aqueous and aluminium hydroxide (AlOH) vaccine formulations results in significant increases in serum IgG anti-45W.

## EXAMPLE 12

## ADJUVANT ACTIVITY OF RECOMBINANT IL-12

An experiment similar to that described in Example 11 for recombinant IL-5, is conducted for the co-expressed recombinant IL-12 35 kDa and 40 kDa polypeptide subunits, produced from the plasmid pCI-neo/IL-12 (Example 7.4), to ascertain the adjuvant potential of recombinant IL-12. Serum antibody levels are quantified by EIA using GST-45W antigen. As in Example 11, adjuvant effects are seen in the PBS and AlOH vaccine groups when approximately at least 10-100μg of recombinant IL-12 is incorporated.

## EXAMPLE 13

## ADJUVANT ACTIVITY OF RECOMBINANT IL-5 AND IL-12 IN COMBINATION

The combination of both IL-5 and IL-12 is studied in AlOH vaccine formulations. Table 4 shows the vaccine formulations used for this experiment. Animals (five per group) are injected i/m in the rear leg on day 0 and receive a second i/m injection in the opposing rear leg on day 28. The cytokines exert synergistic co-adjuvant effects when administered

with AlOH. Antibody titres are elevated significantly compared to titres obtained when AlOH alone is used as adjuvant. The level of antibody obtained with the AlOH-cytokine combination is commensurate with that obtained with Quil A.

**TABLE 4**

**Vaccine formulations comprising combinations of recombinant IL-5 and IL-12 polypeptides**

| VACCINE FORMULATION | $\mu$g of recombinant ovine interleukin | |
|---|---|---|
| | IL-5 | IL-12 |
| 1. AlOH | 0 | 0 |
| 2. AlOH | 10-100 | 0 |
| 3. AlOH | 0 | 10-100 |
| 4. AlOH | 10-100 | 10-100 |
| 5. QUILA | 0 | |
| 6. Controls (no vaccine) | 0 | |

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

(ii) TITLE OF INVENTION: IMMUNE RESPONSE MODULATORS AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 14-JUN-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: AU PN3502/95
(B) FILING DATE: 14-JUN-1995

(A) APPLICATION NUMBER: AU PN6244/95
(B) FILING DATE: 27-OCT-1995

(viii) ATTORNEY/AGENT INFORMATION:
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(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +61 3 9254 2777
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 520 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..441

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CTTTCTTTGC CAAAGGCAAA CGCTGAACAT TTCAGAGTCA AGAGA ATG CAT CTG          54
                                                    Met His Leu
                                                      1

CGT TTG ACC TTG GTA GCT CTT GGA GCT GCC TAT GTT TGT GCC AAT GCT        102
Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys Ala Asn Ala
  5                  10                  15

GTA GAA AGT ACC ATG AAT AGA CTG GTG GCA GAG ACC TTG ACA CTG CTC        150
Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu Thr Leu Leu
 20                  25                  30                  35

TCC ACG CAT CAA ACT CTG CTG ATA GGT GAT GGG AAC TTG ATG ATT CCT        198
Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu Met Ile Pro
                 40                  45                  50

ACT CCT CAG CAT ACA AAT CAC CAA CTA TGC ATT GAA GAA GTC TTT CAG        246
Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu Val Phe Gln
             55                  60                  65

GGA ATA GAC ACA TTG AAG AAT CAA ACT GCA CAA GGG GAT GCT GTG AAA        294
Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp Ala Val Lys
         70                  75                  80

AAA ATA TTC CGA AAC TTG TCT TTA ATA AAA GAA TAC ATA GAC CTC CAA        342
Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile Asp Leu Gln
     85                  90                  95
```

```
AAA AGG AAG TGT GGA GGA GAA AGA TGG AGA GTG AAA CAA TTC CTC GAC      390
Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln Phe Leu Asp
100                 105                 110                 115

TAC CTG CAA GTT TTC CTT GGT GTG ATA AAC ACA GAG TGG ACG ATG GAA      438
Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp Thr Met Glu
                120                 125                 130

AGC TGA GAT CTA CCT CTC TCA CTG TAG TGA AAG TTT CTG GAG GAG GAG      486
Ser  *

AAG GAT GTT TTA ATT GCA GTC AGA ATG AGG GCC A                        520
```

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met His Leu Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys
 1               5                  10                  15

Ala Asn Ala Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu
            20                  25                  30

Thr Leu Leu Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu
        35                  40                  45

Met Ile Pro Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu
    50                  55                  60

Val Phe Gln Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp
65                  70                  75                  80

Ala Val Lys Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile
                85                  90                  95
```

```
Asp Leu Gln Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln
            100                 105                 110

Phe Leu Asp Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp
        115                 120                 125

Thr Met Glu Ser  *
    130
```

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..396

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG CAT CTG CGT TTG ACC TTG GTA GCT CTT GGA GCT GCC TAT GTT TGT      48
Met His Leu Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys
  1               5                  10                  15

GCC AAT GCT GTA GAA AGT ACC ATG AAT AGA CTG GTG GCA GAG ACC TTG      96
Ala Asn Ala Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu
            20                  25                  30

ACA CTG CTC TCC ACG CAT CAA ACT CTG CTG ATA GGT GAT GGG AAC TTG     144
Thr Leu Leu Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu
        35                  40                  45

ATG ATT CCT ACT CCT CAG CAT ACA AAT CAC CAA CTA TGC ATT GAA GAA     192
Met Ile Pro Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu
    50                  55                  60
```

```
GTC TTT CAG GGA ATA GAC ACA TTG AAG AAT CAA ACT GCA CAA GGG GAT      240
Val Phe Gln Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp
 65                  70                  75                  80

GCT GTG AAA AAA ATA TTC CGA AAC TTG TCT TTA ATA AAA GAA TAC ATA      288
Ala Val Lys Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile
                 85                  90                  95

GAC CTC CAA AAA AGG AAG TGT GGA GGA GAA AGA TGG AGA GTG AAA CAA      336
Asp Leu Gln Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln
            100                 105                 110

TTC CTC GAC TAC CTG CAA GTT TTC CTT GGT GTG ATA AAC ACA GAG TGG      384
Phe Leu Asp Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp
        115                 120                 125

ACG ATG GAA AGC TGA                                                  399
Thr Met Glu Ser
    130
```

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met His Leu Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys
 1               5                  10                  15

Ala Asn Ala Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu
            20                  25                  30

Thr Leu Leu Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu
        35                  40                  45

Met Ile Pro Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu
    50                  55                  60
```

```
Val Phe Gln Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp
 65                  70                  75                  80

Ala Val Lys Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile
                 85                  90                  95

Asp Leu Gln Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln
            100                 105                 110

Phe Leu Asp Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp
        115                 120                 125

Thr Met Glu Ser
    130
```

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 554 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA (partial)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
AC CAC CAC CTC AGT TTG GCC AGG AGC CTG CCC ACC ACC ACA GCA GGC      47
   His His Leu Ser Leu Ala Arg Ser Leu Pro Thr Thr Thr Ala Gly
    1               5                  10                  15

CCA GGA AGG AGT TGC CTT GAC TAC TCC CAA AAC CTG CTG AGG GCC GTC      95
Pro Gly Arg Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg Ala Val
                20                  25                  30

AGC AAC ACG CTG CAG AAG GCC AGA CAA ACC CTA GAA TTT TAC TCC TGC     143
Ser Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Ser Cys
            35                  40                  45
```

```
ACT TCT GAG GAG ATT GAT CAT GAA GAT ATC ACC AAA GAT AAA ACC AGC      191
Thr Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser
        50                  55                  60

ACA GTG GAG GCC TGT TTA CCA CTG GAA TTA GCC ACG AAT GAG AGT TGT      239
Thr Val Glu Ala Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu Ser Cys
     65                  70                  75

CTG GCT TCC AGA GAG ACC TCT TTA ATA ACT AAT GGG CAT TGT CTG TCT      287
Leu Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys Leu Ser
 80                  85                  90                  95

TCT GGA AAG ACC TCT TTT ATG ACA ACC CTG TGC CTT AGA AGT ATC TAC      335
Ser Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser Ile Tyr
                100                 105                 110

AAG GAC TTG AAG ATG TAT CAC ATG GAG TTC CAG GCC ATG AAT GCA AAG      383
Lys Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn Ala Lys
            115                 120                 125

CTT CTG ATG GAT CCT AAG AGG CAA GTC TTT CTA GAC CAG AAC ATG CTG      431
Leu Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn Met Leu
        130                 135                 140

GCA GCT ATT GCT GAG CTA ATG CAG GCC CTG AAT TTC GAC AGT GAG ACT      479
Ala Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser Glu Thr
    145                 150                 155

GTG CCA CAG AAA CCC TCC CTG GAA GAA CTG GAT TTT TAT AAG ACA AAA      527
Val Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys Thr Lys
160                 165                 170                 175

GTC AAG CTC TGC ATC CTG CTT CAC GCC                                  554
Val Lys Leu Cys Ile Leu Leu His Ala
                180
```

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 184 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
His His Leu Ser Leu Ala Arg Ser Leu Pro Thr Thr Thr Ala Gly Pro
 1               5                  10                  15

Gly Arg Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg Ala Val Ser
            20                  25                  30

Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Ser Cys Thr
        35                  40                  45

Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr
    50                  55                  60

Val Glu Ala Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu Ser Cys Leu
65                  70                  75                  80

Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys Leu Ser Ser
                85                  90                  95

Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser Ile Tyr Lys
            100                 105                 110

Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn Ala Lys Leu
        115                 120                 125

Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn Met Leu Ala
    130                 135                 140

Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser Glu Thr Val
145                 150                 155                 160

Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys Thr Lys Val
                165                 170                 175
```

Lys Leu Cys Ile Leu Leu His Ala
180

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 666 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..663

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATG TGC CCG CTT CGC AGC CTC CTC CTC ATA TCC ACC CTG GTT CTC CTC      48
Met Cys Pro Leu Arg Ser Leu Leu Leu Ile Ser Thr Leu Val Leu Leu
 1               5                  10                  15

CAC CAC CTG CCC CAC CTC AGT TTG GGC AGG AGC CTG CCC ACC ACC ACA      96
His His Leu Pro His Leu Ser Leu Gly Arg Ser Leu Pro Thr Thr Thr
            20                  25                  30

GCA GGC CCA GGA ACG AGT TGC CTT GAC TAC TCC CAA AAC CTG CTG AGG     144
Ala Gly Pro Gly Thr Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg
        35                  40                  45

GCC GTC AGC AAC ACG CTG CAG AAG GCC AGA CAA ACC CTA GAA TTT TAC     192
Ala Val Ser Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr
    50                  55                  60

TCC TGC ACT TCT GAG GAG ATT GAT CAT GAA GAT TTA ACC AAA GAT AAA     240
Ser Cys Thr Ser Glu Glu Ile Asp His Glu Asp Leu Thr Lys Asp Lys
65                  70                  75                  80

ACC AGC ACA GTG GAG GGC TGT TTA CCA CTG GAA TTA GCC ACG AAT GAG     288
Thr Ser Thr Val Glu Gly Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu
                85                  90                  95
```

```
AGT TGT CTG GCT TCC AGA GAG ACC TCT TTA ATA ACT AAT GGG CAT TGT      336
Ser Cys Leu Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys
            100                 105                 110

CTG TCT CCT GGA AAG ACT TCT TTT ATG ACA ACC CTG TGC CTT AGA AGT      384
Leu Ser Pro Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser
        115                 120                 125

ATC TAC AAG GAC TTG AAG ATG TAT CAC ATG GAG TTC CAG GCC ATG AAT      432
Ile Tyr Lys Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn
    130                 135                 140

GCA AAG CTT CTG ATG GAT CCT AAG AGG CAA GTC TTT CTA GAC CAG AAC      480
Ala Lys Leu Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn
145                 150                 155                 160

ATG CTG GCA GCT ATT GCT GAG CTA ATG CAG GCC CTG AAT TTC GAC AGT      528
Met Leu Ala Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser
                165                 170                 175

GAG ACT GTG CCA CAG AAA CCC TCC CTG GAA GAA CTG GAT TTT TAT AAG      576
Glu Thr Val Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys
            180                 185                 190

ACA AAA ATC AAG CTC TGC ATC CTT CTT CAC GCC TTC AGA ATT CGT GCG      624
Thr Lys Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala
        195                 200                 205

GTG ACC ATC GAC AGA ATG ATG AGC TAT CTG AGT TCT TCC TAG              666
Val Thr Ile Asp Arg Met Met Ser Tyr Leu Ser Ser Ser
    210                 215                 220
```

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 221 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Cys Pro Leu Arg Ser Leu Leu Leu Ile Ser Thr Leu Val Leu Leu
 1               5                  10                  15
```

```
His His Leu Pro His Leu Ser Leu Gly Arg Ser Leu Pro Thr Thr Thr
            20                  25                  30

Ala Gly Pro Gly Thr Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg
        35                  40                  45

Ala Val Ser Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr
    50                  55                  60

Ser Cys Thr Ser Glu Glu Ile Asp His Glu Asp Leu Thr Lys Asp Lys
65                  70                  75                  80

Thr Ser Thr Val Glu Gly Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu
                85                  90                  95

Ser Cys Leu Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys
            100                 105                 110

Leu Ser Pro Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser
        115                 120                 125

Ile Tyr Lys Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn
    130                 135                 140

Ala Lys Leu Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn
145                 150                 155                 160

Met Leu Ala Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser
                165                 170                 175

Glu Thr Val Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys
            180                 185                 190

Thr Lys Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala
        195                 200                 205

Val Thr Ile Asp Arg Met Met Ser Tyr Leu Ser Ser Ser
    210                 215                 220
```

(10) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 984 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..981

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
ATG CAC CCT CAG CAG TTG GTC GTT TCC TGG TTT TCC CTG GTT TTG CTG       48
Met His Pro Gln Gln Leu Val Val Ser Trp Phe Ser Leu Val Leu Leu
 1               5                  10                  15

GCA TCT CCC ATC GTG GCC ATA TGG GAA CTG GAG AAA AAT GTT TAT GTT       96
Ala Ser Pro Ile Val Ala Ile Trp Glu Leu Glu Lys Asn Val Tyr Val
            20                  25                  30

GTA GAA TTG GAT TGG TAT CCT AAT GCT CCT GGA GAA ACA GTG GTC CTC      144
Val Glu Leu Asp Trp Tyr Pro Asn Ala Pro Gly Glu Thr Val Val Leu
        35                  40                  45

ACA TGT GAC ACT CCT GAA GAA GAT GGC ATC ACC TGG ACC TCA GAC CAG      192
Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Ser Asp Gln
    50                  55                  60

AGC AGT GAG GTC TTG GGC TCT GGC AAA ACC TTG ACC ATC CAA GTC AAA      240
Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys
65                  70                  75                  80

GAG TTT GGA GAT GCT GGG CAG TAC ACC TGT CAC AAA GGA GGC GAG GTC      288
Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val
                85                  90                  95

CTG AGT CGT TCA CTC CTC CTG CTG CAC AAA AAG GAA GAT GGA ATT TGG      336
Leu Ser Arg Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp
            100                 105                 110
```

```
TCC ACT GAT ATT TTA AGG GAT CAG AAA GAA CCC AAA GCT AAG AGT TTT      384
Ser Thr Asp Ile Leu Arg Asp Gln Lys Glu Pro Lys Ala Lys Ser Phe
        115                 120                 125

TTA AAA TGT GAG GCA AAG GAT TAT TCT GGA CAC TTC ACC TGC TCG TGG      432
Leu Lys Cys Glu Ala Lys Asp Tyr Ser Gly His Phe Thr Cys Ser Trp
    130                 135                 140

CTG ACA GCA ATC AGT ACT AAT TTG AAA TTC AGT GTC AAA AGC AGC AGA      480
Leu Thr Ala Ile Ser Thr Asn Leu Lys Phe Ser Val Lys Ser Ser Arg
145                 150                 155                 160

GGC TCC TCT GAC CCC CGA GGG GTG ACG TGC GGA GCA GCG TCC CTC TCA      528
Gly Ser Ser Asp Pro Arg Gly Val Thr Cys Gly Ala Ala Ser Leu Ser
                165                 170                 175

GCA GAG AAG GTC AGC ATG GAC CAC AGG GAG TAT AAC AAG TAC ACA GTG      576
Ala Glu Lys Val Ser Met Asp His Arg Glu Tyr Asn Lys Tyr Thr Val
            180                 185                 190

GAG TGT CAG GAG GGC AGC GCC TGC CCA GCC GCT GAG GAG AGC CTG CTT      624
Glu Cys Gln Glu Gly Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Leu
        195                 200                 205

ATT GAG GTC GTG ATG GAA ACT GTG CAC AAG CTC AAG TAT GAA AAC TAC      672
Ile Glu Val Val Met Glu Thr Val His Lys Leu Lys Tyr Glu Asn Tyr
    210                 215                 220

ACC AGC AGC TTC TTC ATC AGG GAC ATC ATC AAA CCA GAC CCA CCC AAG      720
Thr Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys
225                 230                 235                 240

AAC CTG CAA CTG AGA CCA TTA AAG AAT TCT CGG CAG GTG GAG GTC AGC      768
Asn Leu Gln Leu Arg Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser
                245                 250                 255

TGG GAG TAC CCT GAC ACG TGG AGC ACC CCG CAT TCC TAC TTC TCC CTG      816
Trp Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu
            260                 265                 270

ACG TTT TGT GTT CAG GTC CAG GGA AAG AAC AAG AGA GAA AAG AAA CTC      864
Thr Phe Cys Val Gln Val Gln Gly Lys Asn Lys Arg Glu Lys Lys Leu
        275                 280                 285
```

```
TTC ACA GAC CAA ACC TCA GCC AAA GTC ACA TGC CAC AAG GAT GCC AAC        912
Phe Thr Asp Gln Thr Ser Ala Lys Val Thr Cys His Lys Asp Ala Asn
    290                 295                 300

ATC CGC GTG CAA GCC CGG GAC CGC TAC TAC AAC TCA TTC TGG AGT GAA        960
Ile Arg Val Gln Ala Arg Asp Arg Tyr Tyr Asn Ser Phe Trp Ser Glu
305                 310                 315                 320

TGG GCA TCT GTG TCC TGC AGT TAG                                        984
Trp Ala Ser Val Ser Cys Ser
                325
```

(11) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met His Pro Gln Gln Leu Val Val Ser Trp Phe Ser Leu Val Leu Leu
  1               5                  10                  15

Ala Ser Pro Ile Val Ala Ile Trp Glu Leu Glu Lys Asn Val Tyr Val
             20                  25                  30

Val Glu Leu Asp Trp Tyr Pro Asn Ala Pro Gly Glu Thr Val Val Leu
         35                  40                  45

Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Ser Asp Gln
     50                  55                  60

Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys
 65                  70                  75                  80

Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val
                 85                  90                  95

Leu Ser Arg Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp
            100                 105                 110
```

```
Ser Thr Asp Ile Leu Arg Asp Gln Lys Glu Pro Lys Ala Lys Ser Phe
        115                 120                 125

Leu Lys Cys Glu Ala Lys Asp Tyr Ser Gly His Phe Thr Cys Ser Trp
    130                 135                 140

Leu Thr Ala Ile Ser Thr Asn Leu Lys Phe Ser Val Lys Ser Ser Arg
145                 150                 155                 160

Gly Ser Ser Asp Pro Arg Gly Val Thr Cys Gly Ala Ala Ser Leu Ser
                165                 170                 175

Ala Glu Lys Val Ser Met Asp His Arg Glu Tyr Asn Lys Tyr Thr Val
            180                 185                 190

Glu Cys Gln Glu Gly Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Leu
        195                 200                 205

Ile Glu Val Val Met Glu Thr Val His Lys Leu Lys Tyr Glu Asn Tyr
    210                 215                 220

Thr Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys
225                 230                 235                 240

Asn Leu Gln Leu Arg Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser
                245                 250                 255

Trp Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu
            260                 265                 270

Thr Phe Cys Val Gln Val Gln Gly Lys Asn Lys Arg Glu Lys Lys Leu
        275                 280                 285

Phe Thr Asp Gln Thr Ser Ala Lys Val Thr Cys His Lys Asp Ala Asn
    290                 295                 300

Ile Arg Val Gln Ala Arg Asp Arg Tyr Tyr Asn Ser Phe Trp Ser Glu
305                 310                 315                 320

Trp Ala Ser Val Ser Cys Ser
                325
```

(12) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTTTCTTTGC CAAAGGCAAA CGC 23

(13) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGGCCCTCAT TCTCACTGCA 20

(14) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCGGATCCA TGCATCTGCG TTTGACCTTG 30

(15) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCAGCTTTCC ATGCTCCACT C 21

(16) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCGGATCCA CCACCTCAGT TTGGCCAGG 29

(17) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCGGATCCG GCGTGAAGCA GGATGCAGAG 30

(18) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGCCTCGAGA TGTGCCCGCT TCGCAGCCTC 30

(19) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGCGGTACCC TAGGAAGAAC TCAGATAGCT 30

(20) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGCGGATCCA TGCACCCTCA GCAGTTGGTC 30

(21) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGCGTCGACA CTGCAGGACA CAGATGCCCA 30

CLAIMS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a nucleotide sequence encoding an ovine cytokine or a functional or immunologically interactive homologue, analogue or derivative thereof, wherein said cytokine is IL-5.

2. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a complementary to a nucleotide sequence encoding an ovine cytokine or a functional or immunologically interactive homologue, analogue or derivative thereof, wherein said cytokine is IL-12 or a polypeptide subunit of IL-12 as hereinbefore defined.

3. The isolated nucleic acid molecule according to claim 2, wherein the cytokine is a fusion cytokine between different subunits of IL-12.

4. The isolated nucleic acid molecule according to any one of claims 1 to 3 wherein the nucleotide sequence comprises deoxyribonucleotides.

5. The isolated nucleic acid molecule according to claim 4 wherein the nucleotide sequence is a double-stranded cDNA or synthetic DNA molecule.

6. The isolated nucleic acid molecule according to claim 1 or 3 wherein said IL-5 cytokine further comprises a nucleotide sequence set forth in any one of SEQ ID Nos: 1 or 3 or a homologue, analogue or derivative thereof.

7. The isolated nucleic acid molecule according to claim 2 or 3 wherein said IL-12 subunit is a 35 kDa IL-12 polypeptide.

8. The isolated nucleic acid molecule according to claim 7 wherein said IL-12 subunit further comprises a nucleotide sequence set forth in any one of SEQ ID Nos: 5 or 7 or a

homologue, analogue or derivative thereof.

9. The isolated nucleic acid molecule according to claim 2 or 3 wherein said IL-12 subunit is a 40 kDa IL-12 polypeptide.

10. The isolated nucleic acid molecule according to claim 9 wherein said IL-12 subunit further comprises a nucleotide sequence set forth in SEQ ID No: 9 or a homologue, analogue or derivative thereof.

11. An isolated DNA molecule which encodes a molecule having interleukin activity and is capable of hybridising under at least medium stringency conditions as hereinbefore defined to one or more of SEQ ID Nos: 1, 3, 5, 7 or 9 or a complementary sequence or a homologue, analogue or derivative thereof, wherein said interleukin comprises an amino acid sequence which corresponds or is at least 70% identical to all or a functional or immunologically-interactive part of any one of SEQ ID Nos: 2, 4, 6, 8 or 10.

12. The isolated DNA molecule according to claim 11 wherein said interleukin is ovine IL-5.

13. The isolated DNA molecule according to claim 11 wherein said interleukin is ovine IL-12.

14. A genetic construct which comprises a nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes an ovine IL-5 polypeptide or a homologue, analogue or derivative thereof.

15. The genetic construct according to claim 14 wherein said nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID No: 1 or SEQ ID No: 3 or a homologue, analogue or derivative thereof.

16. The genetic construct according to claim 14 wherein said nucleic acid molecule encodes an IL-5 polypeptide comprising an amino acid sequence set forth in SEQ ID No. 2 or 4 or is at least 70% identical thereto.

17. A genetic construct which comprises a nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes an ovine IL-12 polypeptide or a homologue, analogue or derivative thereof.

18. The genetic construct according to claim 17 wherein said nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID No: 5 or SEQ ID No: 7 or a homologue, analogue or derivative thereof.

19. The genetic construct according to claim 17 wherein said nucleic acid molecule encodes an IL-12 polypeptide which has an estimated molecular weight of approximately 35 kDa, as determined using SDS/PAGE.

20. The genetic construct according to claim 19 wherein said nucleic acid molecule encodes an IL-12 polypeptide further comprising a sequence of amino acids set forth in SEQ ID No: 6 or SEQ ID No: 8 or is at least 80% identical thereto.

21. The genetic construct according to claim 17 wherein said nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID No: 9 or a homologue, analogue or derivative thereof.

22. The genetic construct according to claim 17 wherein said nucleic acid molecule encodes an IL-12 polypeptide having an estimated molecular weight of approximately 40 kDa as determined using SDS/PAGE.

23. The genetic construct according to claim 22 wherein said nucleic acid molecule further encodes an IL-12 polypeptide comprising a sequence of amino acids set forth in

SEQ ID No: 10 or is at least 80% identical thereto.

24. A genetic construct which comprises a sequence of nucleotides which is capable of hybridising under at least medium stringency conditions as hereinbefore defined to any one of the ovine IL-5 or IL-12 nucleotide sequences set forth in SEQ ID Nos: 1, 3, 5, 7 or 9 or a complementary sequence or a homologue, analogue or derivative thereof.

25. The genetic construct according to any one of claims 14 to 24 further comprising a promoter sequence operably linked to said nucleic acid molecule or sequence of nucleotides.

26. The genetic construct according to claim 25 wherein said promoter is suitable for expression in a bacterial cell.

27. The genetic construct according to claim 26 wherein said promoter is the *tac* promoter, *lac*2 promoter or phage lambda $\lambda_L$ or $\lambda_R$ promoter sequence.

28. The genetic construct according to claim 25 wherein said promoter is suitable for expression in a eukaryotic cell.

29. A recombinant isolated ovine IL-5 polypeptide or a homologue, analogue or derivative thereof.

30. A recombinant isolated ovine IL-12 polypeptide or a homologue, analogue or derivative thereof.

31. The recombinant polypeptide according to claim 29 or 30 wherein said polypeptide comprises a sequence of amino acids set forth in any one of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto or a derivative thereof.

32. A method for the treatment and/or prophylaxis of a livestock animal which has been

exposed to or infected with a pathogenic organism, said method comprising administering to said animal an immunoresponsive effective amount of ovine IL-5 and IL-12 or a homologue, analogue or derivative thereof for a time and under conditions sufficient to maintain, stimulate or enhance the immunoresponsiveness of said animal.

33. The method of claim 32 wherein the ovine cytokine is a recombinant molecule.

34. The method according to claim 33 wherein the recombinant molecule is according to claim 31.

35. The method according to claim 32 or 33 or 34 wherein the animal is selected from the list comprising sheep, horses, pigs, cows, donkeys, emus, ostriches, alpacas, camels, deer and goats.

36. The method according to claim 35 wherein the animal is a sheep.

37. The method according to claim 35 wherein the animal is a cow.

38. The method according to any one of claims 32 to 37 further comprising the administration of one or more antigens.

39. A vaccine comprising a recombinant ovine IL-5 or IL-12 molecule or a homologue, analogue or derivative thereof and an antigen.

40. The vaccine according to claim 39 wherein the recombinant molecule comprises a sequence of amino acids set forth in any one of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto.

41. The vaccine according to claims 39 or 40 further comprising a pharmaceutically

acceptable carrier or diluent.

42. The vaccine according to any one of claims 39 to 41, suitable for veterinary use.

43. A genetic construct comprising a first nucleotide sequence encoding ovine IL-5 or ovine IL-12 or a derivative thereof and a second nucleotide sequence comprising a delivery vehicle which is capable of controlling replication in a bacterial, yeast, insect, protozoan animal or a mammalian cell.

44. The genetic construct according to claim 43 wherein the first nucleotide sequence comprises s sequence of nucleotides set forth in any one of SEQ ID Nos: 1, 3, 5, 7 or 9 or a homologue, analogue or derivative thereof.

45. The genetic construct according to claim 43 or 44 wherein the first nucleotide sequence is linked to a promoter sequence which is capable of regulating expression of said nucleotide sequence in the same cell in which the delivery vehicle is capable of controlling replication.

46. A delivery cell comprising the genetic construct of claim 45.

47. The delivery cell according to claim 46 wherein said cell is a bacterial cell or an attenuated virus.

48. The method according to claim 32 wherein the step of administering an ovine IL-5 or IL-12 molecule is by means of a genetic construct according to any one of claims 43 to 45 or a delivery cell according to claim 46 or 47.

49. The method according to claim 32 wherein the step of administering an ovine IL-5 or IL-12 molecule is by injection.

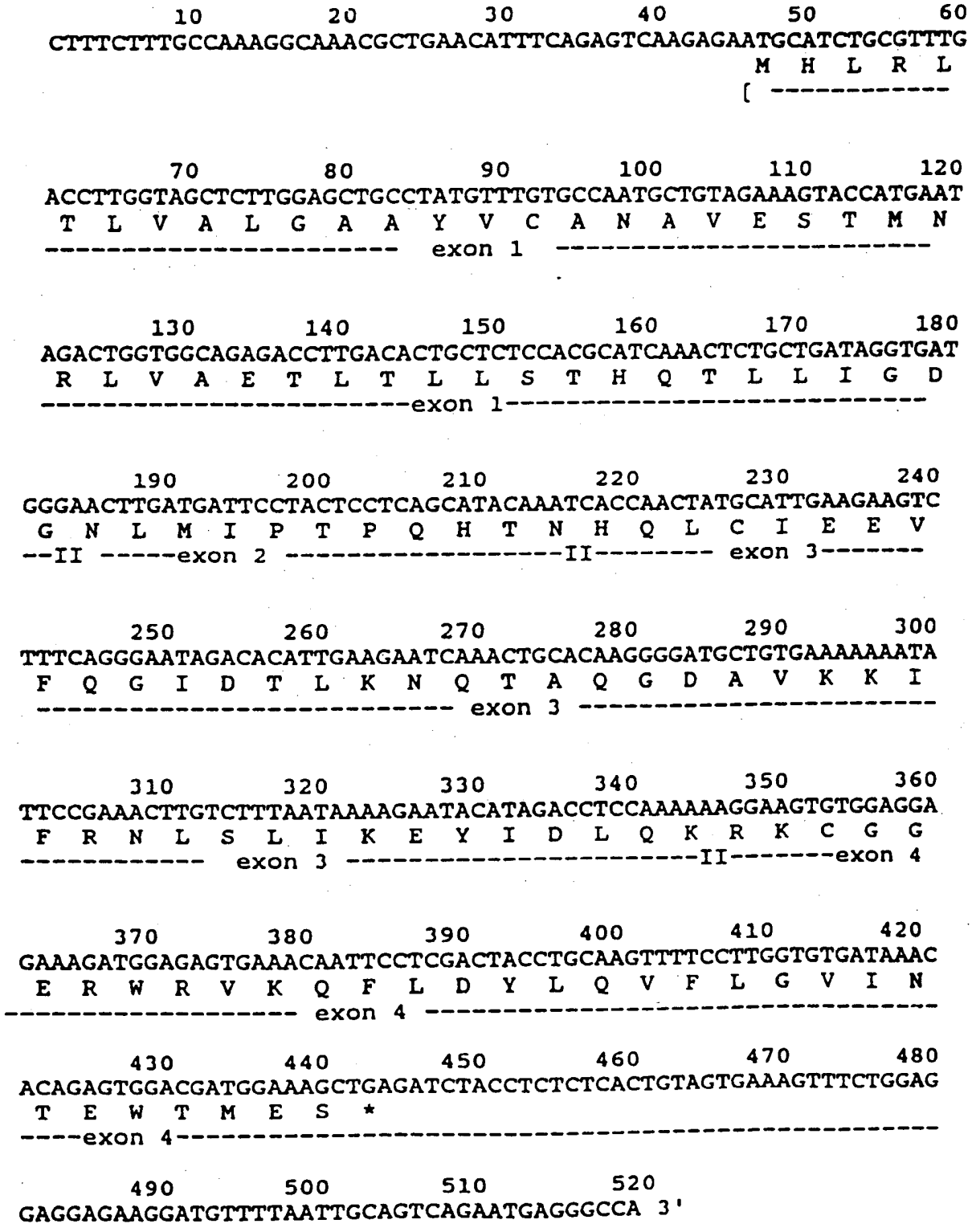
50. A veterinary pharmaceutical composition comprising a recombinant ovine IL-5 or IL-12 polypeptide or a derivative thereof and one or more carriers and/or diluents suitable for veterinary use.

51. The composition according to claim 50 wherein the recombinant ovine IL-5 or IL-12 polypeptide comprises a sequence of amino acids substantially the same as any one of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto.

52. An antibody molecule capable of binding to a recombinant ovine IL-5 or IL-12 polypeptide or a derivative thereof.

53. The antibody molecule according to claim 52 further capable of binding to an IL-5 or IL-12 polypeptide which comprises a sequence of amino acids substantially the same as any of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto.

FIGURE 1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CTTTCTTTGCCAAAGGCAAACGCTGAACATTTCAGAGTCAAGAGAATGCATCTGCGTTTG
M H L R L
[ ----------
70 80 90 100 110 120
ACCTTGGTAGCTCTTGGAGCTGCCTATGTTTGTGCCAATGCTGTAGAAAGTACCATGAAT
T L V A L G A A Y V C A N A V E S T M N
---------- exon 1 ----------
130 140 150 160 170 180
AGACTGGTGGCAGAGACCTTGACACTGCTCTCCACGCATCAAACTCTGCTGATAGGTGAT
R L V A E T L T L L S T H Q T L L I G D
----------exon 1----------
190 200 210 220 230 240
GGGAACTTGATGATTCCTACTCCTCAGCATACAAATCACCAACTATGCATTGAAGAAGTC
G N L M I P T P Q H T N H Q L C I E E V
--II -----exon 2 ----------II-------- exon 3-------
250 260 270 280 290 300
TTTCAGGGAATAGACACATTGAAGAATCAAACTGCACAAGGGGATGCTGTGAAAAAAATA
F Q G I D T L K N Q T A Q G D A V K K I
---------- exon 3 ----------
310 320 330 340 350 360
TTCCGAAACTTGTCTTTAATAAAAGAATACATAGACCTCCAAAAAAGGAAGTGTGGAGGA
F R N L S L I K E Y I D L Q K R K C G G
---------- exon 3 ----------II-------exon 4
370 380 390 400 410 420
GAAAGATGGAGAGTGAAACAATTCCTCGACTACCTGCAAGTTTTCCTTGGTGTGATAAAC
E R W R V K Q F L D Y L Q V F L G V I N
---------- exon 4 ----------
430 440 450 460 470 480
ACAGAGTGGACGATGGAAAGCTGAGATCTACCTCTCTCACTGTAGTGAAAGTTTCTGGAG
T E W T M E S *
----exon 4----------
490 500 510 520
GAGGAGAAGGATGTTTTAATTGCAGTCAGAATGAGGGCCA 3'

FICURE 2

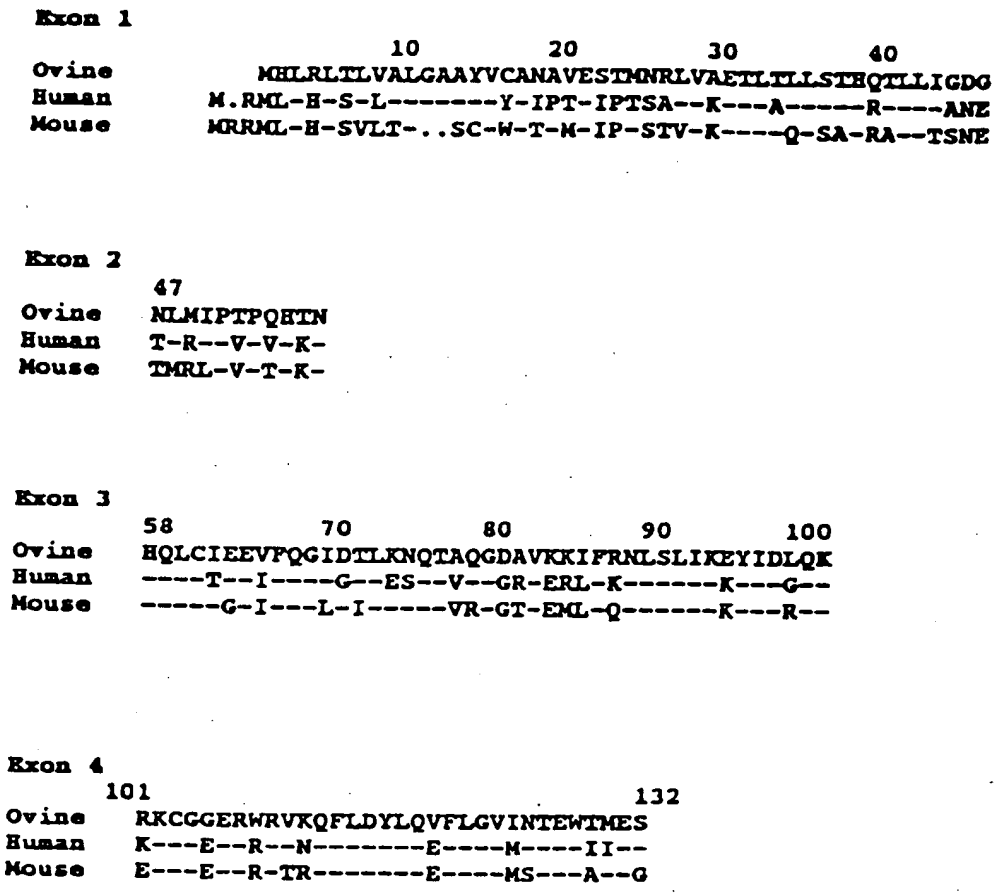
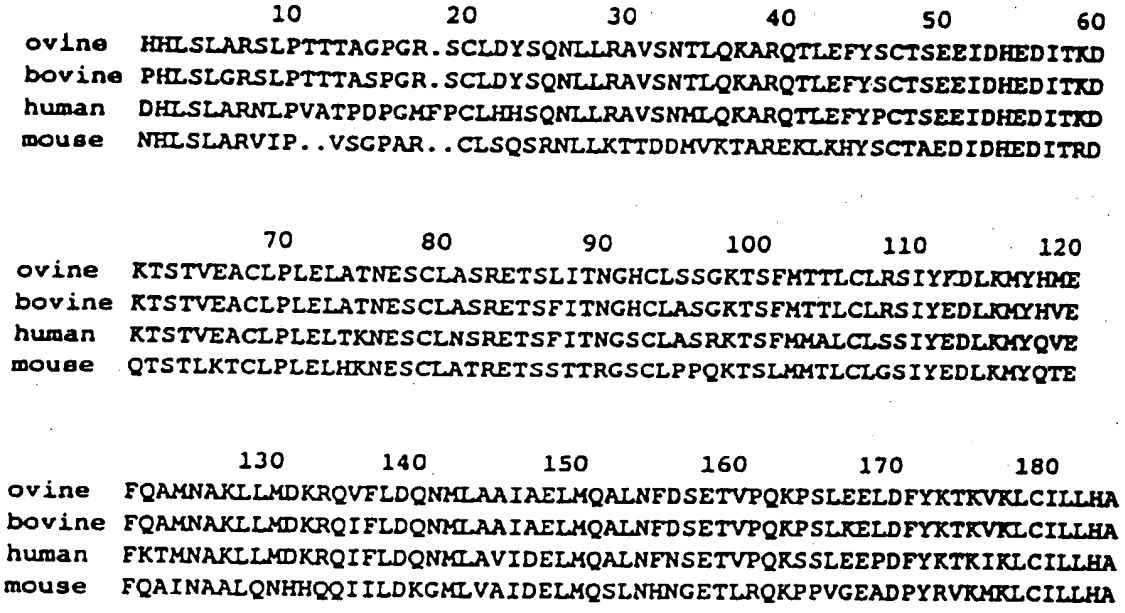

Exon 1
10 20 30 40
Ovine MHLRLTLVALGAAYVCANAVESTMNRLVAETLTLLSTHQTLLIGDG
Human M.RML-H-S-L-------Y-IPT-IPTSA--K---A------R----ANE
Mouse MRRML-H-SVLT-..SC-W-T-M-IP-STV-K----Q-SA-RA--TSNE
Exon 2
47
Ovine NLMIPTPQHTN
Human T-R--V-V-K-
Mouse TMRL-V-T-K-
Exon 3
58 70 80 90 100
Ovine HQLCIEEVFQGIDTLKNQTAQGDAVKKIFRNLSLIKEYIDLQK
Human ----T--I----G--ES--V--GR-ERL-K------K---G--
Mouse -----G-I---L-I-----VR-GT-EML-Q------K---R--
Exon 4
101 132
Ovine RKCGGERWRVKQFLDYLQVFLGVINTEWTMES
Human K---E--R--N-------E----M----II--
Mouse E---E--R-TR-------E----MS---A--G

FIGURE 3


10 20 30 40 50 60
ovine HHLSLARSLPTTTAGPGR.SCLDYSQNLLRAVSNTLQKARQTLEFYSCTSEEIDHEDITKD
bovine PHLSLGRSLPTTTASPGR.SCLDYSQNLLRAVSNTLQKARQTLEFYSCTSEEIDHEDITKD
human DHLSLARNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKD
mouse NHLSLARVIP..VSGPAR..CLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRD
70 80 90 100 110 120
ovine KTSTVEACLPLELATNESCLASRETSLITNGHCLSSGKTSFMTTLCLRSIYEDLKMYHME
bovine KTSTVEACLPLELATNESCLASRETSFITNGHCLASGKTSFMTTLCLRSIYEDLKMYHVE
human KTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVE
mouse QTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSLMMTLCLGSIYEDLKMYQTE
130 140 150 160 170 180
ovine FQAMNAKLLMDKRQVFLDQNMLAAIAELMQALNFDSETVPQKPSLEELDFYKTKVKLCILLHA
bovine FQAMNAKLLMDKRQIFLDQNMLAAIAELMQALNFDSETVPQKPSLKELDFYKTKVKLCILLHA
human FKTMNAKLLMDKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHA
mouse FQAINAALQNHHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHA

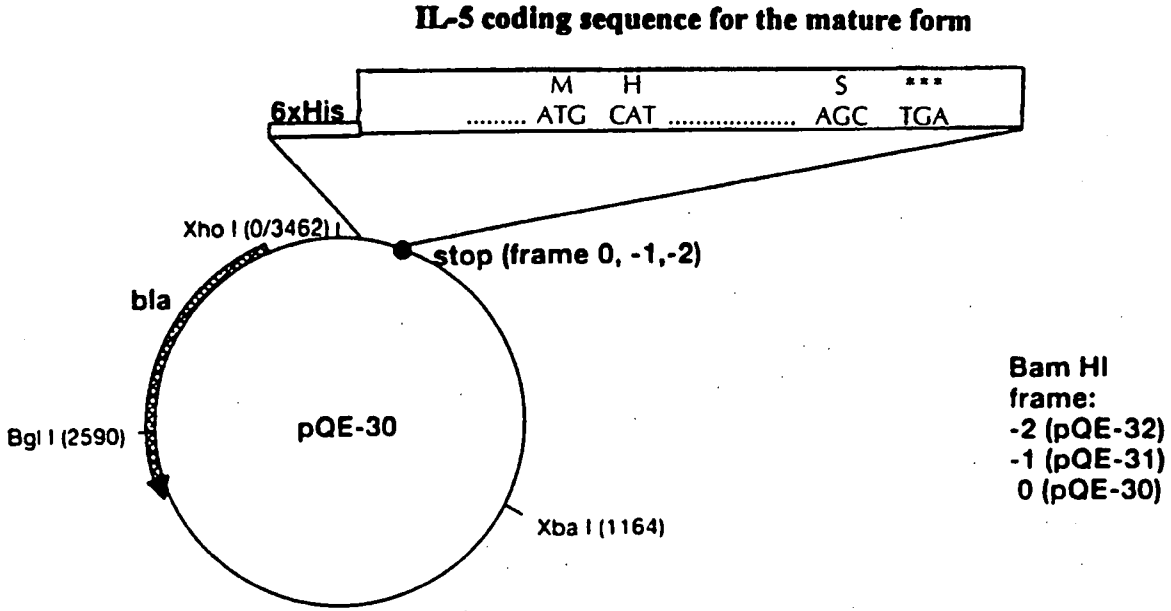
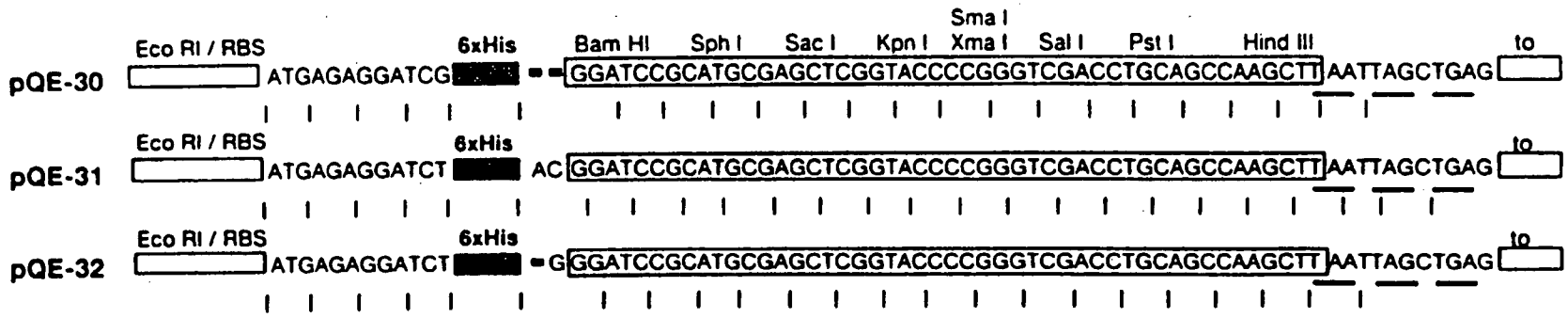

IL-5 coding sequence for the mature form
6xHis
M H S ***
..... ATG CAT ..... AGC TGA
Xho I (0/3462)
stop (frame 0, -1,-2)
bla
pQE-30
Bgl I (2590)
Xba I (1164)
Bam HI
frame:
-2 (pQE-32)
-1 (pQE-31)
0 (pQE-30)

FIGURE 4


Eco RI / RBS
6xHis
Bam HI
Sph I
Sac I
Kpn I
Sma I
Xma I
Sal I
Pst I
Hind III
to
pQE-30
ATGAGAGGATCG
GGATCCGCATGCGAGCTCGGTACCCCGGGTCGACCTGCAGCCAAGCTT
AATTAGCTGAG
pQE-31
ATGAGAGGATCT
AC
pQE-32
G

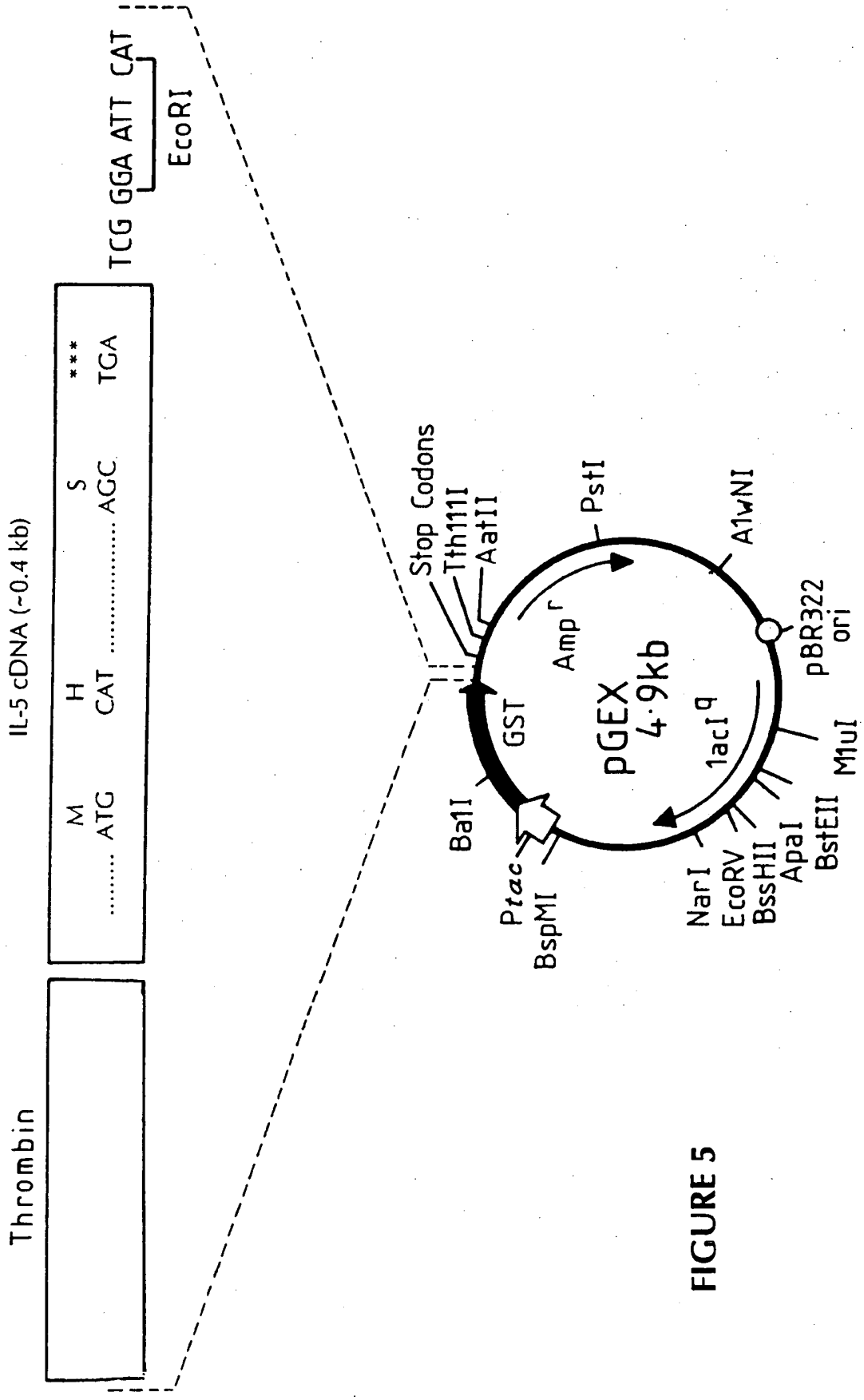

Thrombin
IL-5 cDNA (~0.4 kb)
M
ATG
H
CAT
S
AGC
***
TGA
TCG GGA ATT CAT
EcoRI
Stop Codons
Tth111I
AatII
PstI
A1wNI
pBR322
ori
Amp r
pGEX
4·9kb
GST
1acI q
BamI
Ptac
BspMI
NarI
EcoRV
BssHII
ApaI
BstEII
MluI

FIGURE 5

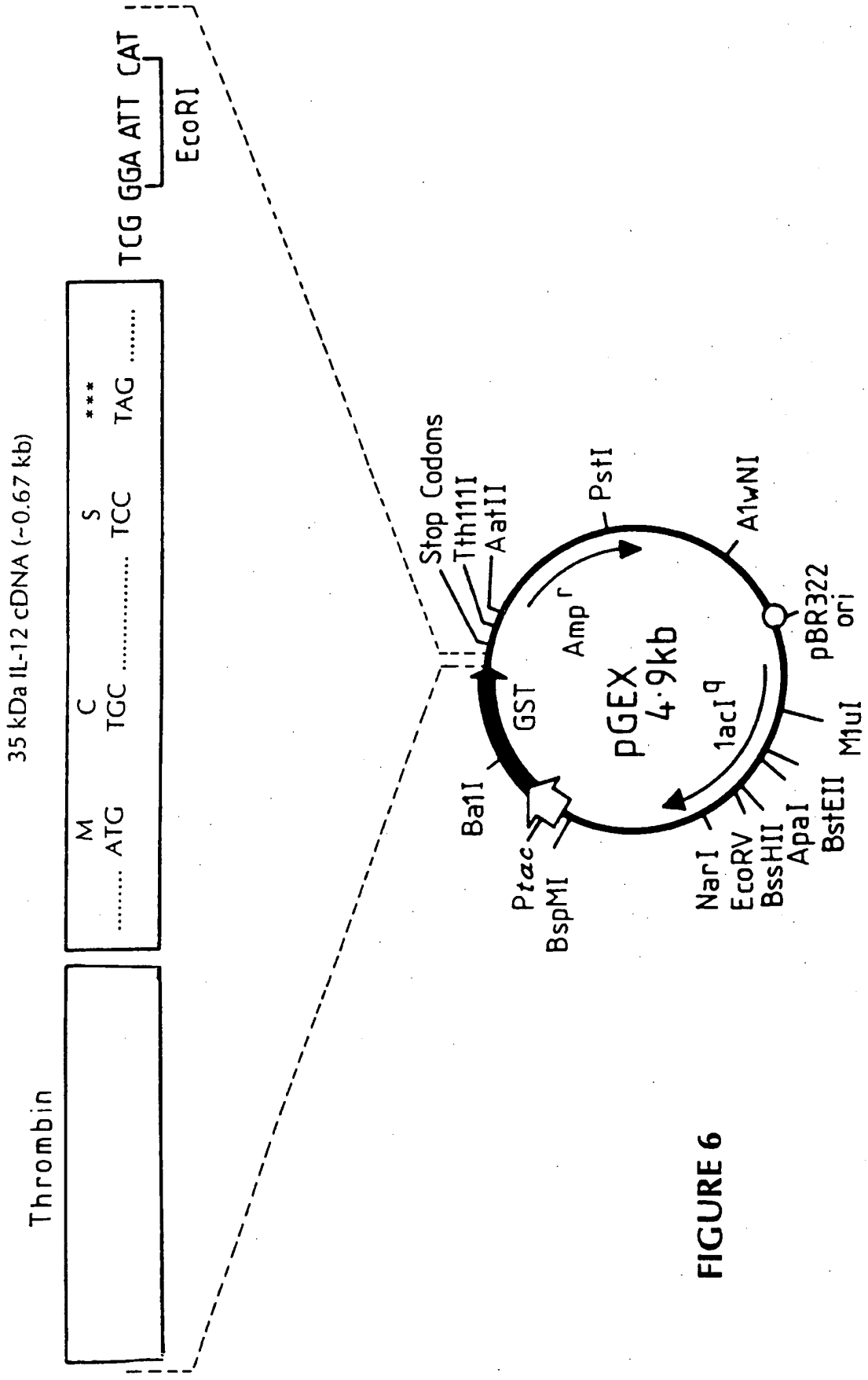

Thrombin
35 kDa IL-12 cDNA (~0.67 kb)
M
ATG
C
TGC
S
TCC
***
TAG
TCG GGA ATT CAT
EcoRI
Stop Codons
Tth111I
AatII
PstI
A1wNI
pBR322 ori
MluI
BstEII
ApaI
BssHII
EcoRV
NarI
Ptac
BspMI
BalI
GST
Amp r
pGEX 4·9kb
lacI q

FIGURE 6

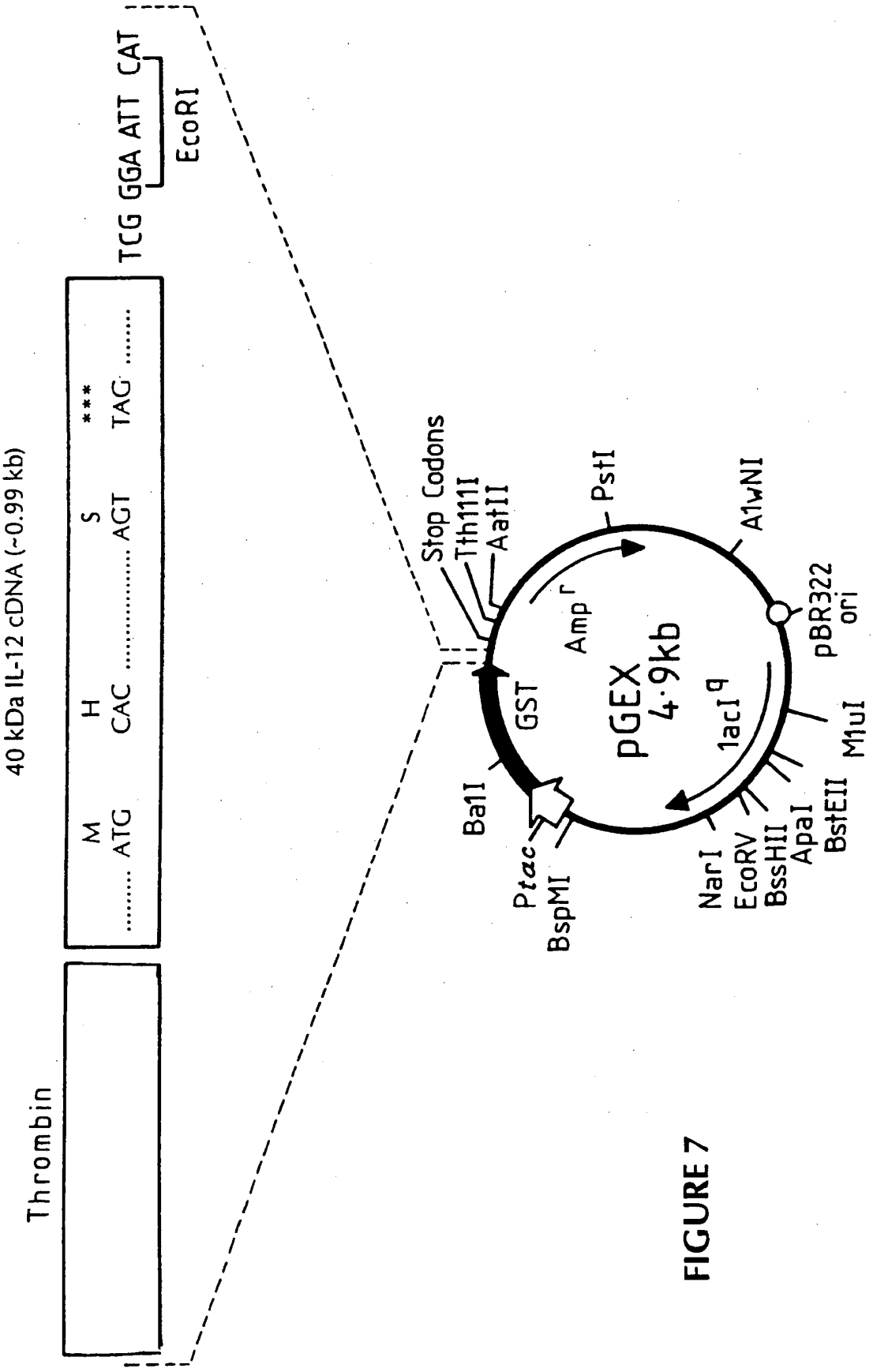

Thrombin
40 kDa IL-12 cDNA (~0.99 kb)
M ATG
H CAC
S AGT
*** TAG
TCG GGA ATT CAT
EcoRI
Stop Codons
Tth111I
AatII
PstI
A1wNI
pBR322 ori
MluI
BstEII
ApaI
BssHII
EcoRV
NarI
BspMI
Ptac
BalI
GST
Amp r
pGEX 4·9kb
1acI q

FIGURE 7

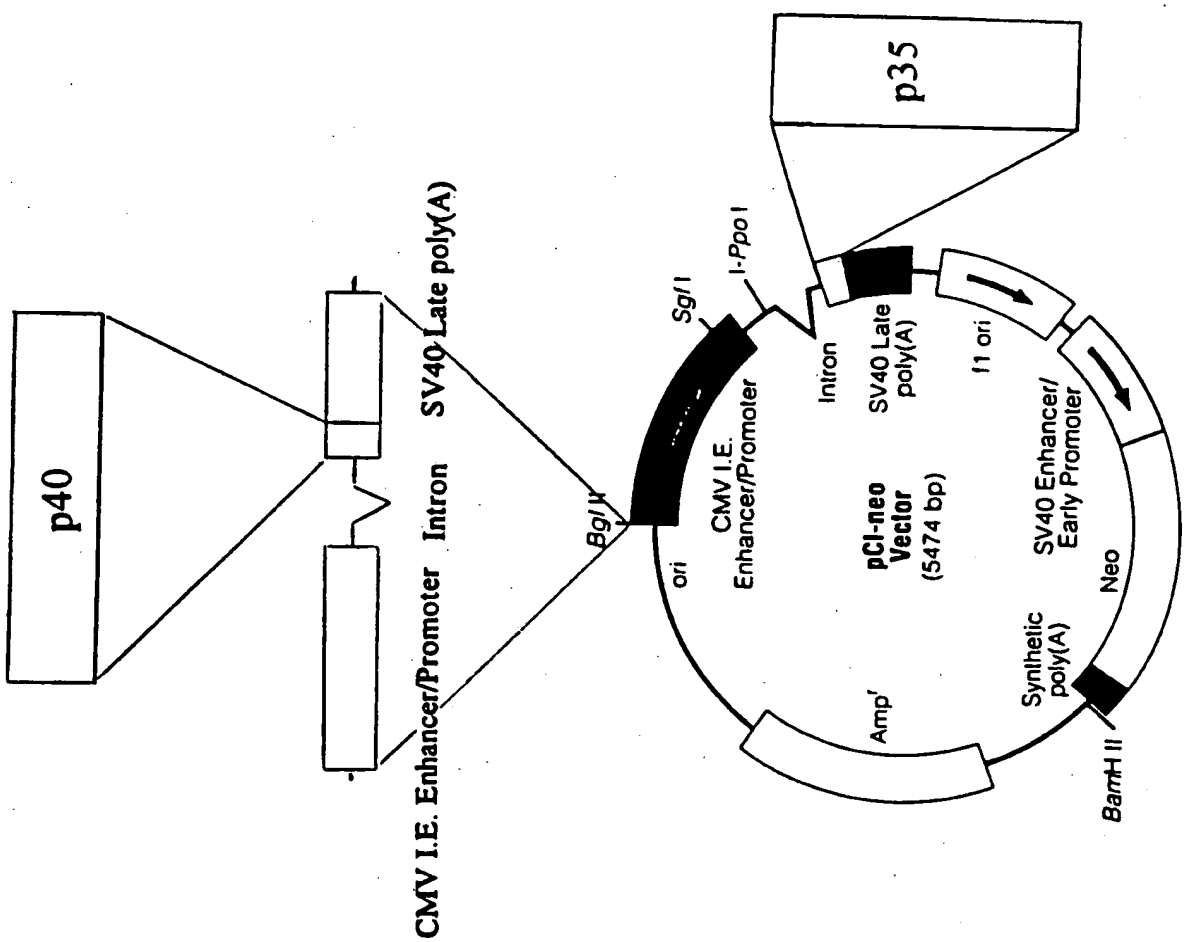

p40
CMV I.E. Enhancer/Promoter
Intron
SV40 Late poly(A)
Bgl II
p35
Sgf I
I-Ppo I
ori
CMV I.E. Enhancer/Promoter
pCI-neo Vector (5474 bp)
Intron
SV40 Late poly(A)
f1 ori
SV40 Enhancer/ Early Promoter
Neo
Synthetic poly(A)
Amp'
BamH II

FIGURE 8

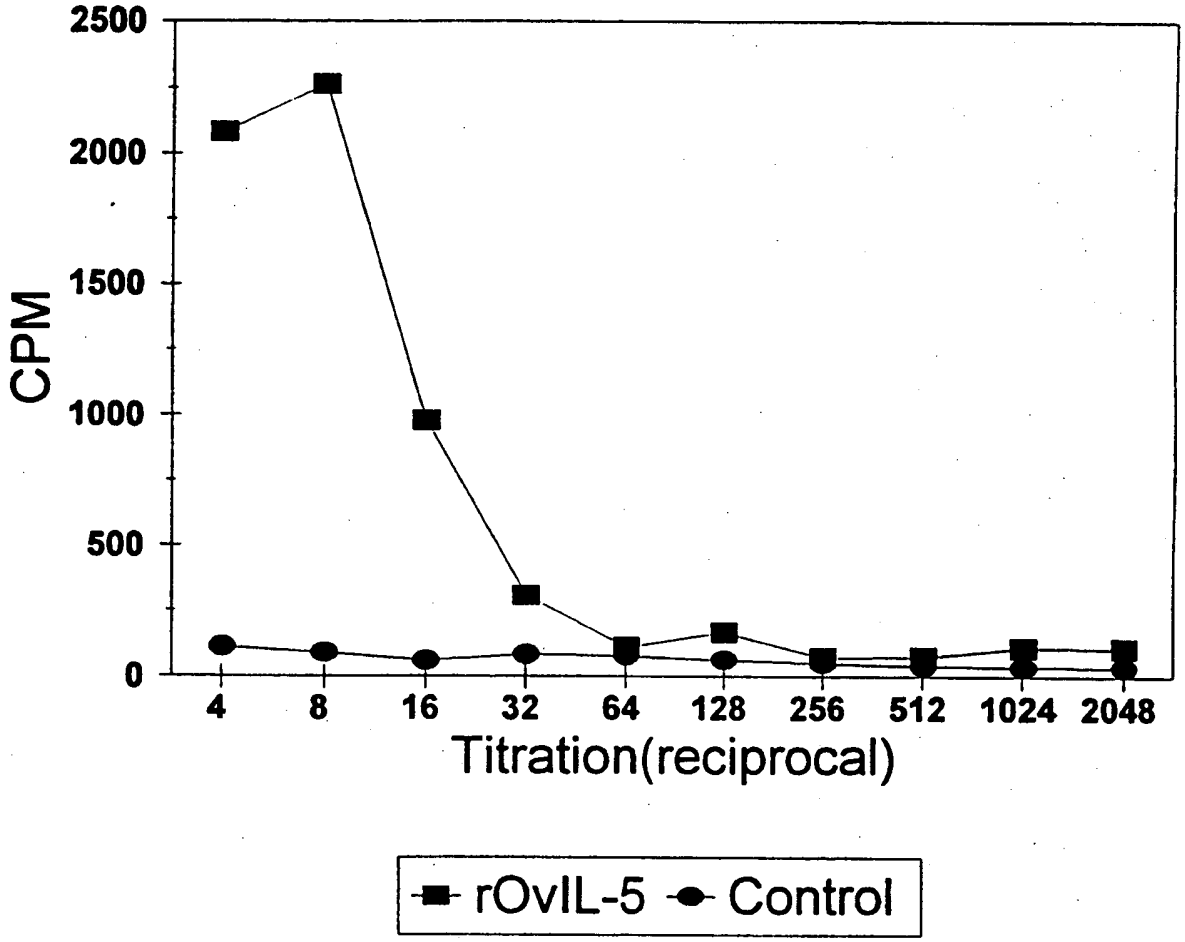

2500
2000
1500
1000
500
0
CPM
4
8
16
32
64
128
256
512
1024
2048
Titration(reciprocal)
rOvIL-5
Control

FIGURE 9

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00360

**A. CLASSIFICATION OF SUBJECT MATTER**

Int Cl[6]: C12N 15/24; C07K 16/24; C12N 15/62, 5/10; A61K 38/20, 48/00.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
WPAT AND CHEM ABS
SEE DETAILS IN ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPM AND JAPIO

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT WPAT, USPM, JAPIO DATA BASES; KEYWORDS: (INTERLEUKIN ()5 OR IL()5 OR IL5 OR EOSINOPHIL() DIFFERENTIATION() FACTOR OR EDF OR EOSINOPHIL() COLONY() STIMULATING() FACTOR OR T() CELL() REPLACING() FACTOR OR EO() CSF OR KHF OR IGA() EF) AND (C12N-015/1C OR A61k/1C).
CHEMICAL ABSTRACTS DATABASE; KEYWORDS: (GENE# OR GENET?)/IT AND INTERLEUKIN()5/IT AND 1991-1996/PY.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>Y | J Biochem (1990), Vol. 107, Pages 292-297. (Minamitake. Y. et al)<br>"Structure of Recombinant Human Interleukin 5 Produced by Chinese Hamster Ovary Cells".<br>See whole Article. | 1,4-6,11,12,<br>14-16, 24-29<br>and 31-53. |

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
13 August 1996

Date of mailing of the international search report
27 AUG 1996

Name and mailing address of the ISA/AU
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AUSTRALIA Facsimile No.: (06) 285 3929

Authorized officer
Arati Sardana
ARATI SARDANA
Telephone No.: (06) 283 2627

Form PCT/ISA/210 (second sheet) (July 1992) copjhw

PCT/INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00360

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | GB 2217328 (British Bio-Technology Limited) published 25 October 1989.<br>See whole document | 1,4-6,11,12,<br>14-16,24-29<br>and 31-53 |
| X | EP 621341 (American Cyanamid Co.) published 26 October 1994.<br>See whole document | 52 and 53 |
| X | Cytokine, (January 1991), Vol. 3, No. 1, Pages 72-81.(Uberla, K. et al.)<br>"The Rat Interleukin-5 Gene: Characterization and Expression by Retroviral gene transfer and Polymerase chain reaction". | 1,4-6,11,12<br>14-16,24-29<br>and 31-53 |
| Y | AU 85278/91 (Commonwealth Scientific and Industrial Research Organization)<br>published 2 April 1992.<br>See whole Article. | 1,4-6,11,12,<br>14-16,24-29<br>and 31-53 |

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00360

**Box 1 Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1, 6, 11, 14-16, 24, 29, 31, 32, 39,40, 44 and 50-53
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Parts of the above claims referring to homologues, derivatives, sequences 70% identical to the parent sequence, and immunologically-interactive parts were found to be unsearchable because they are indeterminate in scope and are not the biological equivalents of ovine IL-5.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

The International Searching Authority has found that there are two inventions:
1. Claims 1, 4-6, 11, 12, 14-16, 24-29 and 31-53 are directed to isolated nucleic acid molecule of Ovine Interleukin 5. Genetic constructs, vaccines, pharmaceutical compositions comprising it. Delivery cells comprising the genetic constructs. Method of treating animals with ovine Interleukin 5 and antibodies to IL-5. This comprises a "first special technical feature".

continued

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:1, 4-6, 11, 12, 14-16, 24-29 and 31-53

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

**Box II continuation**

2. Claims 2-11, 13, 17-28, 30-53 are directed to isolated nucleic acid molecule of ovine Interleukin-12. Genetic constructs, vaccines, pharmaceutical compositions comprising it. Delivery cells comprising the genetic constructs. Method of treating animals with ovine Interleukin-12 and antibodies to IL-12. This comprises a second separate "special technical feature".

The two sets of claims do not share a technical relationship because Interleukin-5 and Interleukin-12 are structurally distinct molecules with different biological functions and unrelated genes.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No.
**PCT/AU 96/00360**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | Patent Family Member | | | | | |
|---|---|---|---|---|---|---|---|
| GB | 2217328 | GB | 8808524 | | | | |
| EP | 621341 | AU | 60591/94 | CA | 2121096 | JP | 7070198 |
| AU | A 91 85278 | NZ | 239791 | WO | 9205255 | | |

END OF ANNEX